IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

NOVOZYMES A/S, Plaintiff v. GENENCOR INTERNATIONAL, INC., and ENZYME DEVELOPMENT CORPORATION Defendants	C.A. No. 05-160-KAJ

PLAINTIFF'S PROPOSED FINDINGS OF FACT AND CONCLUSIONS OF LAW

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NATURE AND STAGE OF PROCEEDINGS

This is an action for patent infringement. Plaintiff Novozymes A/S ("Novozymes") filed a Complaint in this Court (D.I. 1) on March 15, 2005, against defendants Genencor International, Inc. ("Genencor") and Enzyme Development Corporation ("EDC") (collectively "Genencor"). The defendants are accused of infringing Novozymes' U.S. Patent No. 6,867,031 entitled "Amylase Variants" ("the '031 Patent"), by their manufacture, use, offer for sale, and sale of an alpha-amylase product under the trade name Spezyme Ethyl. The defendants each denied infringement and asserted as affirmative defenses that the '031 patent is invalid and is unenforceable (D.I. 89 and 90).

Novozymes moved for a preliminary injunction on June 22, 2005 (D.I. 16). A hearing was held on October 20, 2005, and the Court issued a Memorandum Order on October 24, 2005 denying the preliminary injunction. A "Phase 1" bench trial was held on March 6-9, 2006, on issues of liability, including infringement, validity and unenforceability. A separate "Phase 2" bench trial on issues related to damages and willful infringement has been scheduled to begin on October 10, 2006, if necessary. (D.I. 70 ¶ 8).

Novozymes now submits the following proposed findings of fact and conclusions of law, in support of its claim for judgment that its '031 patent is infringed by Genencor and is not invalid or unenforceable. As shown at trial and set forth herein, the Court should enter judgment: (1) that the '031 patent is infringed; (2) that the '031 patent is not invalid or unenforceable; and (3) permanently enjoining Genencor from the manufacture, use, offer for sale or sale in the United States, or importation into the United States of its Spezyme Ethyl alpha-amylase product, or any other product that infringes the '031 patent.

I. PROPOSED FINDINGS OF FACT

Novozymes presents its proposed findings of fact based on all of the evidence adduced at trial and showing: (a) Genencor's manufacture, use, sale and offer for sale of its Spezyme Ethyl product in the United States infringes claims 1, 3 and 5 of the '031 patent-in-suit; (b) the patent is not invalid; and (c) the patent is not unenforceable.

A. Background

1. The Parties and Their Dispute

1. Plaintiff Novozymes A/S ("Novozymes") is a Danish corporation having a place of business at Krogshoejvej 367, DK-2880 Bagsvaerd, Denmark. **A1003.**[1]

2. Novozymes is the owner, by assignment, of U.S. Patent No. 6,867,031 (the "031 patent") titled "Amylase Variants", which issued on March 15, 2005. **A1005.**

3. Novozymes is the world's largest producer of industrial enzymes. Novozymes' innovations in this area have led to many patentable inventions, including the alpha-amylase variants of the '031 patent. **A5016:24-25.**

4. Defendant Genencor International, Inc. is a Delaware corporation having its principal place of business at 925 Page Mill Road, Palo Alto, California 94304. **A1003.**

5. Defendant Enzyme Development Corporation ("EDC") is a Delaware corporation having its principal place of business at 21 Penn Plaza, New York, New York 10001. **A1003.**

6. Defendants are accused of infringing claims 1, 3 and 5 of U.S. Patent No. 6,867,031 (the '031 patent), owned by plaintiff Novozymes. **TE-100; A1005(P); A1008(A).** More particularly, Novozymes alleges that Defendants' Spezyme Ethyl product is an alpha-amylase enzyme variant having all of the required features of the '031 patent claims.

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[1] Citations are to numbered pages of the trial record, presented as an appendix accompanying the opening post-trial briefs. Line numbers, where applicable, are indicated by a colon. For example, **A5017:5-9** indicates lines 5-9 of page A5017 in the appendix.

7. Defendants answer that they have not infringed any valid or enforceable claim of the '031 patent **(A1703, ¶16)**, and assert that the patent is invalid for failing to satisfy one or more of the requirements of 35 U.S.C. §§ 101, 102, 103, and/or 112. **(A1703 ¶15).**

2. The Technology of the '031 Patent -- Protein Engineering

8. The '031 patent is concerned with engineered variants of a certain type of enzymes: alpha-amylases. All enzymes are proteins that facilitate or catalyze biochemical reactions.

9. All proteins, including alpha-amylases and other enzymes, are chains of amino acids that are joined together by chemical bonds. There are twenty (20) different amino acids (also referred to as "residues," **A5110:9-10**) that occur in nature, and each protein chain includes many of these twenty amino acids assembled in a particular order. The order of amino acids in a protein is known as its "amino acid sequence" **A1004, ¶I**. *See also* **A5019:4-13**.

10. One end of a protein chain of amino acids is called the "N-terminus," and the other end is called the "C-terminus." **A1004**. Scientific nomenclature has assigned a one-letter abbreviation to each of the 20 different natural amino acids. The sequence of a particular protein can be specified by writing the one-letter abbreviation for each amino acid in the order that it appears in the protein chain. **A1004**. For example, R179 indicates the amino acid Arginine at position 179 of an amino acid sequence.

11. A protein's individual characteristics are determined at least in part by the specific "sequence" of the amino acids in the protein chain. These characteristics include, for example, the protein's function (*e.g.*, the particular chemical reaction that the protein facilitates), as well as the conditions under which it optimally performs that function. **A1004**.

12. It can be informative when comparing proteins to compare their respective amino acid sequences. The amino acid sequences are typically "aligned" with one another to produce a juxtaposition showing groups of amino acids that are common to both proteins. **A1005**.

13. The percentage of identical amino acid matches in the "aligned sequences" can then be determined, and reported as the percent "identity" (or, as referred to in the '031 patent, the percent "homology") of the two proteins. **A1005.**

14. When two aligned proteins have different lengths, the consecutive numbered positions for their amino acids may not coincide, because of "gaps" where one sequence has no matching residues in the other. **A5107:19-5108:2; A5108:21-A5108:10** (Dr. Devereux). Thus, a reference sequence can be used for numbering all the sequences in the alignment. **A5142:9-14** (Dr. Arnold).

15. The string of amino acids that make up a protein is folded into a three-dimensional structure. This structure, in turn, determines the properties of the protein. **A5021:1-7** (Dr. Borchert); **A5688:15-22** (Dr. Machius).

16. The linear sequence or string of amino acids of a protein can be called the "primary" structure. **A1004;** *see also* **A5019:4-13.** The folded or three-dimensional structure of a protein can be called its "tertiary" structure. **A5687:13-19.**

17. Protein engineers may also refer to the "secondary" structure of a protein. This term is used to identify local, three-dimensional structural elements or shapes that occur frequently in proteins. These local structures, such as alpha helices and beta sheets, come together in three dimensions to form the specific tertiary structure of the protein.. **A5646:15-5647:11** (Dr. Borchert); **A5687:4-5688:6** (Dr. Machius).

18. The alpha-amylases of the '031 patent are engineered variants. Protein engineering is a science in which the amino acid sequence of a protein is deliberately changed. **A5136:16-21** (Dr. Arnold) The goal of protein engineering is to improve or optimize a protein's properties for an industrial application. ***Id.*** For example, a co-inventor of the '031 patent, Dr. Torben Borchert, explained that, "certain amino acid changes have been introduced in order to make the properties better." **A5017:5-9**. A protein engineer works to "change the amino-acid

sequence of the enzyme in order to make it function better in various industrial processes" (**A5018:14-19**), *e.g.*, at high temperature. **A5018:20-5019:1**.

19. Protein engineering changes include insertions, deletions, and substitutions of amino acids in a protein or enzyme. **A5023:9-13**. Typically, this is done by modifying the gene responsible for the protein at the DNA level (**A5140:4-25** (Dr. Arnold)) "in order to produce a protein that had changes in the amino-acid sequence." **A5204:3-9** (Dr. Alber).

20. Proteins, including alpha-amylases **A5203:23-25** (Dr. Alber), are produced from genes, which are sequences of DNA (not sequences of amino acids). **A5203:23-25** (Dr. Alber). Gene sequences are different than protein sequences. **A5140:4-25** (Dr. Arnold). A triplet of nucleic acids (or nucleotides) in a gene sequence "encodes" each single amino acid (or residue) of the protein. **A5175:20-24** (Dr. Arnold); **A5203:23-5204:3** (Dr. Alber).

21. Proteins are produced from genes by a process called expression. Translation is a step in the expression process of producing the protein. **A5205:18-19; A8881(15)**. The DNA of a gene is used as a template to produce corresponding nucleotide sequences of RNA. The RNA in turn is "translated" to produce a protein product, typically a pre-protein. **A8881(15)**.

22. The gene may first encode a precursor of the actual protein (*i.e.*, a preprotein), which is further processed into its final or mature functional form. Thus, knowledge of the nucleotide sequence of the gene for a protein does not necessarily provide knowledge of the actual amino acid sequence of the final or mature protein. **A5228:11-14** (Dr. Alber). The DNA may encode a precursor or preprotein which is further processed after it is made, typically by cleaving amino acid residues from one or both ends. **A5204:18-5205:3; A5208:19-20** (Dr. Alber).

23. According to Dr. Alber, "the pre-protein is always made from beginning to end by the translation process." **A5208:19-20**. The pre-protein is modified by post-translational modification, to yield the final protein product. **A5204:18-5205:3**. One example of post-translational modification is the removal of the so-called "signal sequence" from the N-terminus

of the pre-protein, which is responsible for the secretion of proteins destined for use outside the cell where they are made. **A5504:13-15** (Dr. Alber). The mature form of a protein is the protein in its active and fully functional form, just as it is when it is doing the work for which it was made. **A5208:17-19.**

24. The actual amino acid sequence of a protein can be verified experimentally, *e.g.*, using mass spectrometry. **A5057:14-24** (Dr. Jorgensen). In particular, mass spectrometry is a tool that can be used to verify that a protein matches a known sequence, *e.g.*, to see if there has been any post-translational modification. ***Id.*** *See also* **A5058:19-22; A5163:3-16** (Dr. Arnold).

25. A wild-type organism is a naturally occurring organism (**A5139:3-4**); a wild-type gene is a naturally found gene that has not been modified (**A5024:2-4** (Dr. Borchert)), and similarly, a wild-type protein is a natural protein which has not been modified. **A6068:2-16.**

26. Variant proteins are typically produced by modifying the gene sequence of a wild-type or unmodified gene for that protein, in order to make a variant gene which then expresses a variant protein. **A5140:19-5141:4** (Dr. Arnold). Thus, the amino acid sequence of the variant protein is altered with respect to the amino acid sequence of the unmodified mature protein. **A5139:4-9.**

27. The unmodified mature protein can be thought of as the starting point or source for protein engineering, by which variants with useful properties can be made. **A5203:2-11** (Dr. Alber) The unmodified mature protein that is altered in order to make a variant protein can be called a "parent" protein. ***Id.***

28. Most often, altering a protein is deleterious and produces undesirable results, such as a loss of function or degradation in properties. "It is unfortunately much easier to damage [a protein] than it is to improve it." **A6115:19-6116:9, A5136:22-5137:7** (Dr. Arnold)

3. <u>Alpha-Amylase Enzymes</u>

29. Novozymes' patent-in-suit, the '031 patent, is concerned with variants of a certain type of enzymes called alpha-amylases. **A1003; A7001 et seq. (TE-100).**

30. Alpha-amylases are specific enzymes that catalyze a biochemical reaction by which starches are degraded into smaller molecules. Starch is a complex molecules composed of many glucose sugar molecules joined together by chemical bonds. Alpha-amylases break apart the starch complexes and convert specific chemical bonds, called the "alpha-1,4-glycoside bonds," between the groups of glucose molecules that make up a starch molecule. **A1003.**

31. Alpha-amylases are produced by many organisms including various species of the bacteria genus *Bacillus*. BAN and BAA are abbreviations for the alpha-amylase from *B. amyloliquifaciens*. BSG and BST are abbreviations for alpha-amylase from *B. stearothermophilus*. BLA is an abbreviation for the alpha-amylase from *B. licheniformis*. **A5577:21-5578:5.**

32. *B. stearothermophilus* is one species of the *Bacillus* genus of bacteria. *Geobacillus stearothermophilus* is a newly accepted name for *B. stearothermophilus*. *B. stearothermophilus* is a soil microbe. **A5022:22-5023:5.** The '031 patent and its claims use the old name. **A1005.**

33. The "activity" of an enzyme is a measure of its ability to catalyze a specific chemical reaction under specified conditions. In the case of alpha-amylases, the alpha-amylase activity is the ability to break down starch. **A5144:10-14.** Enzyme activity, including alpha-amylase activity, can be measured using experiments called "assays." In an assay, the progress of a chemical reaction that is catalyzed by the enzyme is observed under defined conditions, typically using a representative substrate for the reaction, and then observing either degradation of the substrate, or the synthesis of a characteristic reaction product. *See, e.g.* **A5786:14-5788:4; TE100, A7022-23 at 30:55-31:27; 5618:6-5619:22; 5745:23-5746:12.** Enzyme activity can be measured over time, and the activity that is not lost over time can be called "residual activity." **A5786:14-5788:4.** Such assays are often used when testing an enzyme under applicable industrial conditions, such as elevated temperature. ***Id.***

34. Thermostability is the ability of an enzyme to withstand being treated at high temperatures. **A5135:1-4.** Thermostable enzymes, *e.g.* enzyme that can withstand higher temperatures, are desirable for industrial applications such as starch liquefaction in fuel ethanol production. **A5026:1-5027:14; 5029:12-20.** One way to evaluate thermostability is to determine the time needed for an enzyme to lose half of its activity under given temperature conditions. This is called the half life of thermal deactivation or just "half life". **A5786:14-5788:4.**

4. Fuel Ethanol Production

35. Because of their ability to degrade starches into smaller molecules, alpha-amylases are useful in a variety of commercial applications that involve the processing of starches. Alpha-amylases are particular useful in the fuel ethanol industry, where ethanol fuel is produced from starch-rich crops such as corn, barley, and wheat. **A1003.** Alpha-amylases are used to liquefy and reduce the viscosity of starch feedstocks so that they are easier to process in the manufacturing plant. **A1004; TE-100 at 1:35-37.**

36. Fuel ethanol production involves temperatures of 80 degrees C, which are higher than normal for most enzymes found in nature. **A5025:8-15; A5026:5-11.** Thus, thermostable enzymes are particularly desirable for this industry.

B. The Novozymes '031 Patent

37. U.S. Patent No. 6,667,031, "Amylase Variants", was granted on March 15, 2005. The inventors are Henrik Bisgaard-Frantzen, Allan Svendsen, and Torben Borchert. The patent is assigned to Novozymes A/S, the plaintiff in this action. **TE-100 at A7002; A1005.** The application that became the '031 patent was filed as an international application, under the Patent Cooperation Treaty (PCT), on February 5, 1996 as PCT/DK9600056. Priority was claimed to four Danish patent applications, dating back to February 3, 1995. **TE-100 at A7002.**

1. **The Patented Invention**

38. The '031 invention "relates to alpha-amylase variants having improved properties relative to the parent enzyme (e.g. improved thermal and/or oxidation stability and/or reduced calcium ion dependency)". **TE-100, A7008 at 1:21-24**; *see also* ***Id.*** **at 2:60-3, 3:65-4:35.**

39. As stated in the '031 patent, "A goal of the work underlying the present invention was to improve, if possible, the stability of, *inter alia*, particular alpha-amylases which are obtainable from *Bacillus* strains". ***Id.*** **at 3:10-14.**

40. The '031 patent's variant alpha-amylase can be made by genetic engineering. **TE-100, A7008 at 1:26-28** (*e.g.*, DNA constructs encoding variants); also ***Id.*****, 5:5-48.** These techniques are used to modify one or more amino acids of an alpha-amylase by deletion, substitution, or insertion.

41. Particular variants described in the '031 specification are designed to provide improved thermostability. "With respect to increasing the thermal stability of an alpha-amylase variant relative to its parent alpha-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ. ID NO. 1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in . . . SEQ. ID NO. 3 [are] . . . F178, R179, G180, I181, G182 and K183 (SEQ. ID NO. 3)". ***Id.*** **at 9:48-59** (emphasis added). "Particularly interesting pairwise deletions of this type are as follows . . . R179*+G180* . . . (SEQ. ID NO. 3)". ***Id.*** **at 9:62-66.**

42. The same 179,180 deletion is expressly identified as important in the context of achieving reduced calcium dependency. ***Id.*** **at 11:41-65, esp. at 11:57.** Improved or decreased calcium dependency means that the variant exhibits a satisfactory enzyme activity "in the presence of a lower concentration of calcium ion in the extraneous medium" and which, for example, is less sensitive to calcium ion-depleting conditions. ***Id.*** **at 10:40-48.**

43. The claims of the '031 patent are directed to a specific group of the alpha-amylase variants disclosed in the specification. The claimed embodiments are concerned with alpha-amylases that are altered from a *B. stearothermophilus* alpha-amylase and/or that have a defined homology or degree of identity to a *B. stearothermophilus* alpha-amylase parent or the SEQ. ID NO. 3 reference sequence. The claims also specify the deletion of amino acids 179,180 using SEQ. ID NO. 3 for numbering, from among the other deletions, substitutions, and insertions that are disclosed. ***Id.* at 65:30-66:20.**

44. The alpha-amylases claimed in the '031 patent are more thermostable and exhibit satisfactory enzyme activity in the presence of low calcium. ***Id.* at 9:48-59; 9:62-66; 10:40-48; 11:41-65.** This allows the enzyme to function for a longer time in the fuel ethanol process and in other processes and reduces the amount of enzyme needed. It also reduces the use of calcium in the process. **A5025:21-5026:22; A5029:12-20.** This is significant because calcium is necessary or helpful to stabilize a less robust alpha-amylase. Other types of enzymes used in the fuel ethanol industry lose activity in the presence of calcium, so that any calcium that is necessarily present to stabilize alpha-amylase must be removed later in the process. This is a costly and inconvenient step in the fuel ethanol process. **A5026:25-5027:10.**

45. The '031 patent issued from an application that was filed in the United States Patent and Trademark Office (the "PTO") on December 19, 2001: application no. 10/025,648 ("the '648 application"). **TE-100, cover page (A7002).**

46. When Novozymes filed its '648 application, it also submitted a Preliminary Amendment **(TE-101 at A7045 to A7048)** introducing 18 new claims, numbered 30-47, respectively. New claims 30-39 were directed to variant alpha-amylases, whereas claims 40-47 were directed to DNA, vectors, host cells, and methods of expressing the variant alpha-amylases. **TE-101 at A7045 to A7048.** *See also Id.* **at A7621.**

47. Specifically, new claim 30 was independent and specified **(A7045)**:

> [a] variant of a parent alpha-amylase enzyme, wherein said parent alpha-amylase has an amino acid sequence which has at least 80% homology to SEQ. ID NO. 3, and wherein said variant comprises deletions at positions equivalent to positions 179 and 180 in SEQ. ID NO. 3 (using SEQ. ID NO. 3 for numbering).

48. Claims 31-34 all depended directly from claim 30. Claims 31-33 specified that "said parent alpha-amylase has an amino acid sequence which has at least" 85%, 90% or 95% homology, respectively, to SEQ. ID NO. 3. Claim 34 specified that "said variant further comprises amino acid substitutions of a cysteine at positions equivalent to positions 349 and 428 in SEQ. ID NO. 3." **A7045 to A7046.**

49. New claim 35 was also independent and read as follows (**A7046**):

> [a]n isolated alpha-amylase enzyme comprising an amino acid sequence having an amino acid sequence which has at least 80% homology to SEQ. ID NO. 3, modified by having deletions at positions equivalent to positions 179 and 180 in SEQ. ID NO. 3.

50. Claims 36-39 all depended directly from claim 35. Claim 36 specified that "said alpha-amylase enzyme is further modified by having amino acid substitutions of a cysteine at positions equivalent to 349 and 428 in SEQ. ID NO. 3. Claims 37-39 specified that "said alpha-amylase has an amino acid sequence which has at least" 85%, 90% and 95% homology, respectively, to SEQ. ID NO. 3. **A7046**

51. Therefore, by December 19, 2001, Novozymes was pursuing claims for a variant alpha-amylase having deletions at positions 179 and 180, using SEQ. ID NO. 3 for numbering.

52. The PTO issued an Office Action in the '648 application on July 29, 2003 (the "first Office Action"). **TE-101 at A7619 to A7629.** The claims directed to DNA, vectors, host cells, and expression of the variant alpha-amylases – *i.e.*, claims 40-47 – were subject to a restriction requirement and were withdrawn from prosecution. Novozymes elected its claims for the variant alpha-amylase enzymes – *i.e.*, claims 30-39 –for prosecution. **A7621.**

53. The PTO examiner rejected claims 30-39 for indefiniteness under 35 U.S.C. § 112, second paragraph; for failing to comply with the enablement and written description

requirements under 35 U.S.C. § 112, first paragraph; and as obvious under 35 U.S.C. § 103(a). **A7619 to A7629.**

54. The PTO examiner suggested that the rejections under 35 U.S.C. § 112, second paragraph could be overcome if claim 36 were amended to recite "[a]n isolated alpha-amylase comprising an alpha-amylase of claim 35 having amino acid substitutions of cysteine at positions equivalent to 349 and 428 of SEQ. ID NO. 3." **A7622.**

55. With respect to the rejections under 35 U.S.C. § 112, first paragraph, the PTO examiner stated that these rejections could be overcome by amending claim 30 to specify "wherein said variant has at least 80% sequence identity to SEQ. ID NO. 3." **A7627.**

56. The PTO examiner relied upon two references to reject Novozymes' claims as obvious under 35 U.S.C. § 103(a): (i) an article published in the November 15, 1989 issue of the *Journal of Biological Chemistry* by Yutaka Suzuki *et al.*, titled "Amino Acid Residues Stabilizing a Bacillus α [Alpha]-Amylase against Irreversible Thermoinactivation" ("Suzuki" **TE-115**); and (ii) International Patent Publication No. WO 95/10603 by Bisgard-Frantzen *et al.*, titled "Amylase Variants" and dated April 20, 1995 (the "Bisgard-Frantzen" **TE-177**). **A1007, ¶AA.**

57. Suzuki **(TE-11, A8233)** describes mutants or variants of *Bacillus amyloliquefaciens* alpha-amylase enzyme ("BAN"). Suzuki says that some of these mutant alpha-amylases have improved thermostability compared to the native or wild-type *Bacillus amyloliquefaciens* alpha-amylase from which they were derived. **A1007, ¶BB.**

58. Bisgard-Frantzen **(TE-177, A8403)** describes variant alpha-amylase enzymes from three *Bacillus* species: *B. amyloliquefaciens* ("BAN"), *B. stearothermophilus* ("BSG"), and *B. licheniformis* ("BLA"); and teaches that these alpha-amylases have highly homologous amino acid sequences. **A1007, ¶CC.**

59. When making the obviousness rejection, the PTO examiner stated that "it would have been obvious to one of ordinary skill in the art to introduce the mutations disclosed by Suzuki *et al.* into the corresponding positions of *Bacillus stearothermophilus* [alpha]-amylase in

order to produce a homologous [alpha]-amylase which would have been reasonably expected to have similar improved properties in view of the known homology between these [alpha]-amylases." **TE-100 at A7628;** *see also* **A1008, ¶EE.**

60. Novozymes' in-house patent attorney, Jason Garbell, filed an "Amendment Under 37 C.F.R. 1.111" on January 14, 2004, responding to the rejections in the first Office Action. **TE-101, A7633 - A7637.** Mr. Garbell amended independent claim 30 as the PTO examiner had recommended, to specify that "said variant has at least 80% identity to said parent alpha-amylase" **A7634.** Mr. Garbell also amended independent claims 30 and 35 to include the limitations of dependent claims 34 and 36, respectively. **A7636.**

61. The PTO issued another Office Action on April 6, 2004 (the "Second Office Action"), responding to Mr. Garbell's amendments. **TE-101, A7717 - A7727.** The PTO examiner withdrew her previous rejections for indefiniteness under 35 U.S.C. § 112, second paragraph and for obviousness under 35 U.S.C. § 103(a). However, the Examiner maintained the previous rejections under 35 U.S.C. § 112, first paragraph. ***Id.***

62. The PTO examiner stated, in particular, that "the specification, while being enabling for [an alpha]-amylase having at least 90% homology to SEQ. ID NO. 3 and comprising a deletion of residues equivalent to 179 and 180 of SEQ. ID NO. 3 ... does not reasonably provide enablement for any variant of a parent [alpha]-amylase having at least 80% homology to SEQ. ID NO. 3...." **A7721.**

63. On September 3, 2004, Mr. Garbell and co-inventor Dr. Borchert attended a personal interview with the PTO Examiner. Novozymes presented a draft Declaration by Dr. Borchert and a set of proposed claim amendments. The PTO examiner indicated that the declaration appeared to show unexpected results that would overcome the previous obviousness rejection. **TE-101 at A7735, A7798 to A7799.** *See also* **A5013:12-22.**

64. Mr. Garbell, on September 7, 2004, submitted another "Amendment Under 37 C.F.R. 1.111" and an executed "Declaration of Torben V. Borchert Under 37 C.F.R. 1.132,"

dated September 6, 2004, responding to the previous Office Action. **TE-101 at A7733 to A7738, A7739 to A7756.** In that amendment, Mr. Garbell canceled all of the original claims and introduced new claims 48-52, specifying variants of a *Bacillus stearothermophilus* alpha-amylase or variants homologous to a reference sequence, SEQ. ID NO. 3. ***Id.*** Those new claims are identical to claims 1-5 in the '031 patent.

65. The PTO examiner withdrew all rejections and issued a "Notice of Allowance and Fee(s) Due" on September 21, 2004. Claims 48-52 were allowed. **TE-101 at A7791 to A7797.** *See*, in particular, at **A7795.**

66. In her statement of reasons for allowance (**TE-101 at A7796**), the PTO Examiner stated that:

> the declaration of Dr. Torben Borchert submitted 9/7/04 establishes that the claimed variants exhibit unexpectedly large increases in thermostability when compared to the increases in thermostability obtained for the corresponding mutations taught by Suzuki et al. As such the claimed variants are non-obvious over the prior art.

2. Claim Construction

67. The '031 patent claims variant alpha-amylases with deletions of the amino acids at positions equivalent to positions 179 and 180, using SEQ. ID NO. 3 from the '031 patent for numbering purposes. **A1005.**

68. The '031 patent claims are directed to alpha-amylase enzymes, *i.e.*, proteins which are defined by their amino acid sequences. More specifically, the '031 patent claims compare two proteins, one of which is a variant of the other, which is a parent. Although a variant is typically made by genetic manipulation, e.g. by altering the DNA of a parent, the claims in suit are directed to the final protein product, not to altered DNA. **A5204:12-17.** Proteins are made within cells when the DNA of a gene that encodes for the protein is expressed. According to the claims, the resulting variant protein is compared another protein. **A5141:2-5.** The DNA used to express these proteins is not compared.

69. The predicted amino acid sequence of a protein, based on a given gene or presumptive coding DNA, is not necessarily the sequence of the functional, mature protein actually produced by the cell. **TE-200; A5163:17-5164:17.** The claims of the '031 patent do not depend on these DNA issues.

(a) Claim 1

70. Claim 1 of the '031 patent reads as follows **(TE-100 at A7040)**:

> A variant of a parent *Bacillus stearothermophilus* alpha-amylase, wherein the variant has an amino acid sequence which has at least 95% homology to the parent *Bacillus stearothermophilus* alpha-amylase and comprises a deletion of amino acids 179 an 180, using SEQ. ID NO. 3 for numbering, and wherein the variant has alpha-amylase activity.

71. Claim 1 specifies a "variant" having an amino acid sequence that differs from the amino acid sequence of an unaltered *B. stearothermophilus* alpha-amylase "parent." The claim requires comparing the amino acid sequence of the variant protein with the amino acid sequence of the parent protein, specifically a *B. stearothermophilus* parent. SEQ. ID NO. 3 is expressly used "for numbering" as a reference sequence, to number the amino acid residues of the parent and variant, in order to determine whether the specified 179,180 residues are deleted. This can be done by aligning the parent and variant with SEQ. ID NO. 3, using known methods as specified in the patent. **A1005.** SEQ. ID NO. 3 is not required for determining percent homology and it is not necessarily the parent. **A1005; A7040; A5145:13-20.**

72. Claim 1 requires that amino acids 179 and 180 in the parent are not present in the variant.

73. Claim 1 also specifies at least a 95% percent homology comparison between the parent and variant. Again, this comparison in claim 1 is between the variant and the parent protein; it is not a comparison between the variant and SEQ. ID NO. 3. That is, SEQ. ID NO. 3 can be, but is not required to be, a parent of claim 1.

(i) "Variant"

74. Claim 1 provides a "variant" of a parent *B. stearothermophilus* alpha-amylase. **A7040**. The '031 specification states that a "variant" is the result of the deletion, substitution, or insertion of amino acids relative to a parent alpha-amylase. Specifically, "[t]he variants of the invention are variants in which: (a) at least one amino acid residue of the parent alpha-amylase has been deleted; and/or (b) at least one amino acid residue of the parent alpha-amylase has been replaced (i.e. substituted) by a different amino acid residue; and/or (c) at least one amino acid residue has been inserted relative to the parent alpha-amylase." **TE-100 at A7009, 3:59-67**. *See also* **A5138:23-5138:20**. This is done in order to provide modified properties "relative to the present enzyme." **TE-100 at A7008**.

75. "[T]he term 'variant' is used interchangeably with the term 'mutant'." **TE-100 at A7010, 5:49-50.**

76. A variant is the product of human manipulation of a protein sequence, identified as a parent protein. It is a modified version of a parent; it is derived from a parent. **A5138:23-5139:9** (Dr. Arnold); **A5203:2-11** (Dr. Alber). This is how the term is used in the patent, and it is how the term would have been understood by one of ordinary skill in the art of protein engineering in 1995, in light of the '031 patent and its file history. **A5171:17-5172:25.**

77. In the '031 patent specification, a "variant" is consistently a protein that has an amino acid sequence which is modified relative to a parent enzyme. **TE-100 at A7008, 1:20-25; 2:61-63**. A variant enzyme differs from the parent "by judicial modification of one or more amino acid residues in various regions of the amino acid sequence of the parent alpha-amylase" (***Id.* **at 7009, 3:17-25**). *See also, Id.* **at 5:49-57** (variant "deriving from an unmodified parent alpha-amylase").

(ii) "Parent"

78. Claim 1 provides a variant of a "parent" *B. stearothermophilus* alpha-amylase. **A7040**. The '031 specification states that a parent is an antecedent protein which is altered to provide a variant protein. For example: (a) "The present invention relates to alpha-amylase variants having improved properties relative to the parent enzyme (e.g. improved thermal and/or oxidation stability and/or reduced calcium ion dependency)" (**TE-100, A7008 at 1:21-24**); (b) "An object of the present invention is to provide alpha-amylase variants which -- relative to their parent alpha-amylase -- possess improved properties of importance, ... e.g. increased thermal stability" (**TE-100, A7008 at 2:61-65**); (c) "A goal of the work underlying the present invention was to improve, if possible, the stability of, inter alia, particular alpha-amylases which are obtainable from Bacillus strains" (**TE-100, A7008 at 3:30-35**). *See also* (**TE-100, A7009 at 3:18-23**).

79. "The DNA sequence encoding a parent alpha-amylase may be isolated from any cell or microorganism producing the alpha-amylase in question, using various methods well known in the art." **TE-100, A7008 at 12:49-52; 13:24-25.** A parent protein is made from a predecessor gene relative to the variant, often a naturally occurring (*i.e.*, a "wild-type") gene. Its protein sequence is unaltered relative to the variant. **A5150:13-5151:7; TE-100, A7016 at 17:18-19.**

80. Non-limiting examples of suitable "parent" proteins are also disclosed. In a "first aspect" of the invention the parent is one which has the amino acid sequence of SEQ. ID NO. 1, 2, 3, or 7; or is at least 80% homologous to one of these protein sequences, cross-reacts with an antibody raised against an alpha-amylase with one of these protein sequences, or is encoded by a DNA that hybridizes to the same probe as a DNA sequence encoding one of these protein sequences. **TE-100, A7009 at 3:25-42.** The exemplary parents of SEQ. ID NO. 1, 2, 3, and 7 are pre-existing alpha-amylases, isolated and purified from nature. **TE-100, A7011 at 7:18-40; A7025 at 36:7-8** (wild-type gene for SEQ. ID NO. 2).

81. There are also "further criteria for identifying a suitable parent alpha-amylase" candidate. **TE-100, A7011 at 7:52-67.** The parent proteins, modified to produce improved variants, are "alpha-amylases which are obtainable from *Bacillus* strains and which themselves have been selected on the basis of their starch removal performance." **TE-100, A7009 at 3:10-14.** Thus, the examples in the specification are expressly not the only parents and do not limit the ordinary meaning of "parent" in the context of the invention, *i.e.* in the field of protein engineering. The "parent" in claim 1 is not limited to SEQ. ID NO. 3, which is recited in the claim as a reference "for numbering" not as the parent sequence. **A5640:10-17; A5742:15-20; A5142:22-5143:10; A5639:11-18.**

82. The '031 patent specification also identifies preferred mutations which can be made in representative parents, to produce variants of the invention. In doing so, the specification clarifies that similar mutations can be made to any other suitable parents, in corresponding fashion, i.e. "in equivalent positions in the sequence of another alpha-amylase meeting one of the other criteria for a parent alpha-amylase" **(TE-100, A7012 at 9:5-8)**, and specifically "in the context of the invention" **(*Id.* at 9:33-35)**.

83. According to the specification **(*Id.* at 9:19-27)** (emphasis added):

> [T]he term "equivalent position" denotes a position which, on the basis of an alignment of the amino acid sequence of the parent alpha-amylase in question with the "reference" alpha-amylase amino acid sequence in question (for example the sequence shown in SEQ. ID NO. 1) so as to achieve juxtapositioning of amino acid residues/regions which are common to both, corresponds most closely to (e.g. is occupied by the same amino acid residue as) a particular position in the reference sequence in question.

See also, ***Id.* at 10:1-3, 14-17, 36-37** (deleting certain amino acids for thermostability, "or equivalents ... in another alpha-amylase meeting the requirements of a parent alpha-amylase in the context of the invention"). ***Id.* at 10:52-54, 11:8-11, 11:35-36, 11:63-65** (same for calcium dependency).

84. These disclosures make plain that certain sequences given in the specification (*e.g.* SEQ. ID NOs. 1, 2, 3, and 7) are "reference" sequences. Although these proteins may be used as suitable parents, they are not the only parents as the term is used in the '031 patent.

85. As used in the patent, a "parent" is a protein that is made from a naturally-occurring (*i.e.*, "wild-type") gene, meaning that the parent is found in nature and is not the product of protein engineering. **A5150:13-5151:7** (Dr. Arnold). The specification indicates that native enzymes are contemplated as parents. *See e.g.*, **TE-100, A7016 at 17:18-19**, "Naturally occurring enzymes may be genetically modified by random or site directed mutagenesis as described above." *See also*, **TE-100, A7009 at 3:10-14** (parents are "alpha-amylases which are obtainable from *Bacillus* strains"). The parent sequence referred to in the claims is a protein sequence. **A5175:6-7** (Dr. Arnold). Genencor's expert, Dr. Alber, agrees (**A5203:5-11**).

> Q. Have you formed any opinion about what that phrase would mean to one of ordinary skill in the protein engineering art as of 1995?
>
> A. Yes. The parent is the source or ancestor of protein and the variant is something derived from that parent. And the variant contains changes, which in the field are defined as substitutions, insertions and deletions.

This ordinary meaning of "parent" is the same meaning used throughout the '031 patent,

(iii) "*B. Stearothermophilus* Alpha-Amylase"

86. In addition to reciting a "variant of a parent," claim 1 of the '031 patent specifies that the parent is more specifically a "*B. stearothermophilus* alpha-amylase." **TE-100, A7040.** An alpha-amylase is an enzyme having alpha-amylase activity: the ability to catalyze reactions which break down and liquefy starch. A5144:10-19. *B. stearothermophilus* is a species of bacteria found in soil that produces alpha-amylase. **A5139:23-5140:14.**

87. There are additional species of *Bacillus*, such as *B. amyloliquifaciens* and *B. licheniformis*. **A1007; A5577:21-5578:4.** Though related, *e.g.*, in an evolutionary sense, different *Bacillus* species are different organisms. They have different genes and they produce different alpha-amylase enzymes. ***Id.***

88. The actual amino acid sequence of a protein, as made in working form by cells which produce that protein, can be determined from a protein sample obtained from those cells, using known analytical methods. ***See e.g.*** **5058:5-16; A5060:16-18.**

89. As used in the patent, "*B. stearothermophilus* alpha-amylase" refers to the functional and completely processed wild-type enzyme, as provided by cells which produce that enzyme from a *B. stearothermophilus* alpha-amylase gene. This amino acid sequence has not been altered by protein engineering. This amino acid sequence is the actual sequence of the enzyme, which can be experimentally determined. **A5139:1-7** (Dr. Arnold). It is not a predicted or estimated sequence based on translating, *ex vivo*, the DNA of a *B. stearothermophilus* alpha-amylase gene according to the genetic code. **A5228:11-14** (Dr. Alber). It is also not the sequence of an intermediate protein, such as the preprotein product of a gene, before expression and post-translational processing are complete. **A5208:19-20; A5204:18-5205:3.**

(iv) Percent Homology

90. Claim 1 provides that the variant of the invention, "has an amino acid sequence which has at least 95% homology to the parent *B. stearothermophilus* alpha-amylase." **TE-100, A7040.**

91. The term "percent homology" does not have a clear meaning in the art. **A5129:5-16.** However, the '031 patent expressly discusses the meaning of "percent homology" in the context of the invention, as follows (**TE-100, A7009, 4:36-49**):

> An amino acid sequence is considered to be X % homologous to the parent alpha-amylase if a comparison of the respective amino acid sequences, performed via known algorithms, such as the one described by Lipman and Pearson in Science 227 (1985) p. 1435, reveals an identity of X %. The GAP computer program from the GCG package, version 7.3 (June 1993), may suitably be used, employing default values for GAP penalties [Genetic Computer Group (1991) Programme Manual for the GCG Package, version 7, 575 Science Drive, Madison, Wis., USA 53711].

92. The '031 patent equates "percent homology" with the more precise term, "percent identity", and provides specific instructions for making this comparison between two amino acid sequences. ***Id.***; *See also,* **A5525:5-9** (Dr. Alber).

93. Determining percent homology is a two step process. First, two sequences to be compared are "aligned" according to known algorithms, which maximize their overlap in order to find the most corresponding matches. **A1005.** These algorithms can be used, for example, to determine whether specific amino acid differences between two sequences can be interpreted conceptually as deletions, substitutions, or insertions. **A5107:21-5108:2; A5110:13-21.** Second, after alignment, the number of matches are counted, also according to a known algorithm, to determine percent homology. **A1005(O)** As with any "percent," this calculation compares two values, a numerator and a denominator, to produce a fraction which is multiplied by 100% to give "percent homology." **A5110:25-5111:6.**

94. The '031 patent specifically and unambiguously directs the artisan to align and calculate percent homology with the algorithms as described by the Lipman & Pearson paper and the GAP GCG program, using its default parameters. **TE-100; A7009:4:36-49.**

95. The Lipman and Pearson paper cited in the '031 patent is an algorithm for aligning sequences, not for calculating percent homology. **A5235:1-20** (Dr. Alber). The GAP program was the first program by which one could use such algorithms automatically to perform an optimal alignment of two sequences. **A5107:11-17.** The GAP program takes, as input, two sequences (e.g. amino acid sequences) and aligns them optimally. **A5108:13-17.**

96. "An optimal alignment is an attempt to find the relationship between two sequences that maximizes the number of matches but it tolerates some GAPs [*sic*:gaps]. That means the two sequences are slightly different lengths but basically matching it can introduce small different lengths in order to make an optimal sequences that maximizes the number of matches." **A5107:21-108:2.** A gap is a place in one sequence that has no corresponding part in the other sequence. **A5110:13-21.**

97. The GAP GCG program also calculates percent identity or percent homology. A5110:22-25. This percent homology determination asks the question: "How much of one sequence is present in the other sequence?" **A5118:3-10**. Percent identity is a percent comparison of the number of matches between two sequences. The GAP GCG program does not treat as a match, a gap in the amino acid sequence of one protein aligned to another. "Percent identify takes the sum of all the matching residues where there is a corresponding part in both sequences." **A5111:2-4**. The number of exact matches between two amino acid sequences (the numerator) is divided by the number of these amino acid residues where that are residues present in both sequences (the denominator), times 100%. **A5111:4-6**; *see also* **A5111:10-5112:12**.

98. This percent identity calculation was added to the GAP program before 1995 (**A5115:14-21**), because GAP users asked for this type of calculation to be added. **A5115:5-9**. This was the way users wanted to see percent identity calculated, "because it simply doesn't have a clean definition if you allow gaps." **A5115:7-19**. "The problem with gaps is that they are things where on one sequence there is nothing which corresponds on the other. So they have, they have very little mathematical understandability. You can [*sic*: cannot][2] quantitate something that represents the similarity between something that is and something that isn't." **A5114:23-5115:3**.

99. The '031 patent plainly discloses the GAP program for calculating percent homology. **A5141:21-5142:3; A5146:14-5147:1** ("The patent clearly states that the GAP program of GCG may suitably be used. That leads you to a particular calculation of that percent identity.") Genencor's expert, Dr. Alber, did not know of other programs, *e.g.*, "GAP Huang" or "Align," prior to the litigation, and he did not use them. The program he regularly used in his research was GAP GCG, as in the '031 patent. **A5531:17-21**. Dr. Huang, the author of the GAP Huang program, testified that if other programs (like GAP CGC) worked to provide an optimized alignment, there was no need to use his GAP Huang program **A5573:12-15**, which was

[2] It is clear from the context that this is a typographical error in the trial transcript.

specifically designed as a back-up to handle very difficult alignments with short sequences and long gaps. **A5571:17-20**. Here, the patent unambiguously specifies a customary way of calculating percent homology, well-understood by protein engineers at the time of the invention. The patent does not direct the use of other different or contradictory calculations or computer programs. The patent gives clear guidance, and there is no good reason to look elsewhere.

100. John Deveraux was the principal author of the early GCG package releases. **A5103:2-16.** He testified that the GCG package was first introduced in 1981, was first sold in 1982, and by 2000, had 10 major numbered releases. **A5103:17-5104:11.** The GCG package allows a user to edit, manage, publish, store, and find patterns in and make comparisons between two DNA sequences or two amino acid sequences. **A5104:12-5105:4; A5108:3-17.** GCG was the most widely used sequence comparison package from at least 1986 to 2000 and was a standard in the industry. **A5105:5-5106:1**; **A6107:13-6108:12** (Dr. Zeikus). The 1984 Deveraux *et al.* paper discusses the CGC programs and has been cited more than 14,000 times by other authors. **A5106:9-22.** It has been labeled the fifth most cited paper in all of science. **A5105:23-5107:5.**

101. Therefore, "percent homology", as defined in the '031 patent, means a percent identity calculation according to the standard method known in the art at the time of the invention and embodied in the GAP GCG program, whereby the number of exactly matching amino acid residues in two sequences (the numerator) is compared to the total number of residue positions that are present in both sequences (the denominator), expressed as a percent. Gaps are not included. Positions in the alignment where amino acids are not present in both sequences (they are found in one sequence but not the other) are not counted in the denominator for this calculation. This definition is based on the intrinsic evidence in the patent itself and the stated definition therein, and is supported by evidence of how a protein engineer in 1995 would understand the disclosure.

(v) "A deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering"

102. Each variant of claim 1, compared to its parent, also "comprises a deletion of amino acids 179 and 180 using SEQ. ID NO. 3 for numbering." **TE-100, A7040.**

103. Protein sequences and genetic information sequences in U.S. patents are typically assigned consecutive sequence identification numbers ("SEQ. ID NOs") beginning with SEQ. ID NO. 1, for ease of reference in the patent. The patent then issues with a "sequence listing" setting forth each protein and genetic information sequence by its SEQ. ID NO. *See* 37 C.F.R. § 1.821. SEQ. ID NO. 3 is the amino acid sequence of a preferred patent *B. stearothermophilus* alpha-amylase that is given in the '031 patent's Sequence Listing. **TE-101, A7030-7032.** *See, also,* ***Id.*** at 7:32-35.

104. "Using SEQ. ID NO. 3 for numbering" means that SEQ. ID NO. 3 is expressly a reference sequence (it is not claimed as the parent). This means that the positions of amino acids in an alignment (between parent and variant) are assigned according to corresponding positions (residue numbers) in SEQ. ID NO. 3. *See* TE-100 at 9:19-27. According to claim 1, the amino acids of a parent sequence that align with positions 179 and 180 of SEQ. ID NO. 3 are present in the parent sequence but are not present in the aligned variant sequence. **A5142:5-23; A5143:3-10; A5639:5-18.**

(vi) Alpha-Amylase Activity

105. Claim 1 specifies that the variant has alpha-amylase activity. This means that the variant is capable of catalyzing the breakdown and liquefaction of starch. **A1003-1004.**

(b) Claim 3

106. Claim 3 of the '031 patent recites: (**A1005; TE-100, A7040**):

> 3. A variant alpha-amylase, wherein the variant has at least 95% homology to SEQ. ID NO. 3 and comprises a deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering and wherein the variant has alpha-amylase activity.

107. The term "variant" has the same meaning in claim 3 as in claim 1. *See,* **§II.B.2(a)(i)**, above. However, claim 3 specifies a "variant alpha-amylase," rather than a "variant of a parent *B. stearothermophilus* alpha-amylase enzyme." **TE-100, A7040**. This means that the variant of claim 3 is an altered alpha-amylase enzyme, but it is not required to have a parent from any specific genus or species (*B. stearothermophilus* or otherwise) for comparison with the variant. Instead, the sequence of the variant is compared with SEQ. ID NO. 3. **A1005; A5145:21-25.**

108. The term "percent homology" has the same meaning in claim 3 as in claim 1. *See,* **§II.B.2.(a)(iv)**, above. However, the comparison in claim 3 is specifically to SEQ. ID NO. 3. **A1005; A5145:21-25**.

109. The language "comprises a deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering" has the same meaning in claim 3 as in claim 1. *See,* **§II.B.2(a)(v)**, above.

110. The language "wherein the variant has alpha-amylase activity" has the same meaning in claim 3 as in claim 1. *See,* **§II.B.2(a)(vi)**, above.

111. Claim 3 does not identify a parent, nor does it require the use any genus or species of parent, nor is it necessary to know a parent sequence, since the amino acid sequence of the variant itself is compared directly to SEQ. ID NO. 3 as set forth in the patent. **TE-100, A7030-7032, 45:49**.

112. In sum, claim 3 directly compares the amino acid sequence of the variant protein with the amino acid sequence of SEQ. ID NO. 3. **A5145:16-25**. SEQ. ID NO. 3 is used as a reference sequence for numbering, and it is also used for an alignment with the variant to determine: (a) percent homology and (b) whether the residues at positions 179 and 180 are deleted.

(c) **Claim 5**

113. Claim 5 of the '031 patent recites a variant of a *Bacillus stearothermophilus* alpha-amylase, wherein the alpha-amylase variant consists of a deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering. **A1006**. Claim 5 reads as follows (**TE-100, A7040**):

> 5. A variant of a *Bacillus stearothermophilus* alpha-amylase, wherein the alpha-amylase variant consists of a deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering.

114. The term "variant" has the same meaning in claim 5 as in claim 1. *See,* **§II.B.2(a)(i)**, above.

115. The language "*Bacillus stearothermophilus* alpha-amylase" has the same meaning in claim 5 as in claim 1. *See,* **§II.B.2(a)(iii)**, above.

116. The language "a variant of a *Bacillus stearothermophilus* alpha-amylase" means that the variant is derived from a *Bacillus stearothermophilus* alpha-amylase, *i.e.*, it is originally of *Bacillus stearothermophilus* origin. **A5147:4-11** (Dr. Arnold)

117. The language "a deletion of amino acids 179 and 180, using SEQ. ID NO. 3 for numbering" has the same meaning in claim 5 as in claim 1. *See,* **§II.B.2(a)(ii)**, above. However, claim 5 uses the language "consists of a deletion of amino acids 179 and 180." **A7040**. This means that, whatever *Bacillus stearothermophilus* alpha-amylase is altered to make the variant, the only difference between this *B. stearothermophilus* alpha-amylase and the claimed variant is the deletion of the two amino acids at positions 179 and 180, "using SEQ. ID NO. 3 for numbering." **A5147:12-23** (Dr. Arnold).

C. **The Accused Spezyme Ethyl Product**

118. Novozymes has accused Genencor's Spezyme Ethyl product of infringing claims 1, 3, and 5 of the '031 patent. **A1008; TE-100, A7040**.

119. Genencor was aware of the prosecution leading to the '031 patent, including the pending claims directed to the subject matter now in issue. Novozymes paid the PTO issue fee on September 29, 2004. On that same day, Novozymes gave Genencor a copy of the allowed claims

and notified Genencor of its belief that these claims would be infringed by Genencor's Spezyme Ethyl product upon grant of the patent. **A5014:2-14**. Genencor ignored this warning and proceeded at its own risk. ***Id.;*** **A5664:7-21**. Novozymes brought this action on March 15, 2005, the same day that the '031 patent issued. **A1501; TE-100, A7002**.

120. It is stipulated that defendant Genencor International, Inc. (a) has made, used, offered for sale, and/or sold in the United States or imported into the United States; (b) continues to make, use, offer for sale, and/or sell in the United States or import into the United States; (c) has induced others to use, offer for sale, and/or sell in the United States or import into the United States; (d) and continues to induce others to use, offer for sale, and/or sell in the United States or import into the United States, an alpha-amylase product under the tradename Spezyme Ethyl. **A1006, ¶V.**

121. It is stipulated that defendant EDC is a United States distributor for Spezyme Ethyl alpha-amylase; and has used, offered for sale and/or sold in the United States or imported into the United States, and continues to use, offer for sale, and/or sell in the United States or import into the United States, the Spezyme Ethyl alpha-amylase product. **A1006, ¶W.**

122. It is stipulated that Genencor began selling Spezyme Ethyl in the United States by April of 2004. **A1006, ¶X.**

123. Since its April 2004 launch, Spezyme Ethyl has enjoyed great success. **A5048:14-5049:24**. Genencor's total annual sales of Spezyme Ethyl have almost tripled from 2004 to 2005. **A1006, ¶Y.** Genencor sells Spezyme Ethyl for use in fuel ethanol production. **TE-134, A8355**.

124. Spezyme Ethyl is an engineered variant of a wild-type *B. stearothermophilus* parent enzyme. As Genencor admits: "The alpha-amylase from which SPEZYME Ethyl was derived is the alpha-amylase of *Bacillus stearothermophilus* strain ASP154, ATCC deposit no. 39,709. SPEZYME Ethyl has alpha-amylase activity." **TE-194, A8521**.

125. This same ATCC 39,709 wild-type *B. stearothermophilus* alpha-amylase has also been called "GZYME G997" or simply "G997". Genencor International, Inc. has sold G997. **A5045:16-19; TE-161, A8366** ("Commercially available G997").

126. Genencor also calls Spezyme Ethyl "EBS2" **A5032:4-18** (Crabb). According to work done by Genencor's Judy Chang in 2004, "EBS2 [Spezyme Ethyl] is a recombinant alpha-amylase derived from *Geobacillus stearothermophilus* [*i.e., Bacillus stearothermophilus*]" **TE-161, A8365.** EBS2 [Spezyme Ethyl] "differs from the wild-type s*tearothermophilus* enzyme in two amino acid deletions, the arginine and glycine at positions 181 and 182." **TE-161, A8365.** This 181,182 numbering of the amino acid positions is for alignment with *B. licheniformis* alpha-amylase. ***Id.*, A8365, n.1**. "When aligned against the wild-type *stearothermophilus* alpha-amylase, the [deleted] amino acids are found at positions 179 and 180." ***Id.*** *See also*, **A5167:10-21** (Dr. Arnold); **A5258:10-5259:7**.

127. Thus, Spezyme Ethyl is derived from, and is a variant of, a wild-type *B. stearothermophilus* alpha-amylase, G997. **A5039:12-5040:8, A5045:16-19; A5046:10-13; TE-194, A8521.** Wild-type G997 is the parent of the Spezyme Ethyl alpha-amylase. ***Id***; **A5148:8-5149:18**. The difference between variant Spezyme Ethyl and the G997 parent is an engineered deletion of the amino acids at positions 179 and 180 of the parent *B. stearothermophilus* alpha-amylase. **TE-161, A8365; A8369** (deletions confirmed); **A5161:25-5162:22** (Dr. Arnold); **A5259:23-5260:3, A5260:21-5261:5**.

128. It is stipulated that Genencor's accused Spezyme Ethyl alpha-amylase is a protein of 484 amino acids with the following amino acid sequence (**A1006-1007**):

AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGIT
ALWLPPAYKGTSRSDVGYGVYDLYDLGEFNQKGTVRTKY
GTKAQYLQAIQAAHAAGMQVYADVVFDHKGGADGTEWV
DAVEVNPSDRNQEISGTYQIQAWTKFDFPGRGNTYSSFKW
RWYHFDGVDWDESRKLSRIYKFIGKAWDWEVDTENGNYD
YLMYADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDA
VKHIKFSFFPDWLSYVRSQTGKPLFTVGEYWSYDINKLHNY
ITKTNGTMSLFDAPLHNKFYTASKSGGAFDMRTLMTNTLM
KDQPTLAVTFVDNHDTEPGQALQSWVDPWFKPLAYAFILT

RQEGYPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGT
QHDYLDHSDIIGWTREGVTEKPGSGLAALITDGPGGSKWM
YVGKQHAGKVFYDLTGNRSDTVTINSDGWGEFKVNGGSV
SVWVPRKTT

See also, **A8519**.

129. Novozymes obtained and analyzed samples of G997 and Spezyme Ethyl. The analysis was conducted by Dr. Christian Jorgensen. Dr. Jorgensen received a Ph.D. in molecular biology from Odense University, which is now known as the University of Southern Denmark. **A5055:5-14**. Odense University has a world renowned research group in mass spectrometry analysis. **A5055:15-5056:19**. Dr. Jorgensen is the head of Novozymes' protein characterization group. **A5054:5-12**; **A5055:5-14**. His group supports the entire Novozymes organization with biomolecular analysis of biopolymers (*i.e.*, polymers produced by living organisms), including proteins. **A5054:17-55:4**.

130. Dr. Jorgensen regularly analyzes, characterizes, and identifies proteins, including determining the identification and order (the sequence) of amino acids, and the molecular weights of proteins. **A5058:5-16**. He has done this with thousands of proteins. **A5060:16-18**. He uses techniques such as mass spectrometry, which measures the molecular weight of molecules and which can help to confirm whether a determined amino acid sequence for a protein is correct. **A5057:14-5058-4; A5058:17-22; A5060:5-15**.

131. Dr. Jorgensen, on or about November 4, 2005 ("2005-11-04"), was given a stable liquid sample of G997 for analysis. **A5060:19-62:6; TE-211, A8543.** He inspected the sample and found that it was suitable for analysis. **A5062:7-64:7; A5093:1-13**.

132. Dr. Jorgensen's analysis of G997 (**TE-206, A8537-40; A5065:16-23**) identified two proteins in the sample: (a) a single *B. stearothermophilus* catalase enzyme weighing 55kD; and (b) a single *B. stearothermophilus* alpha-amylase enzyme weighing 58kD. **A5069:18-25; A5070:6-11**. The catalase present in the G997 sample is a common background protein; it is not a contaminant or an alpha-amylase degradation product. **A5077:14-23; A5093:25-5094:6**.

133. The alpha-amylase in the sample was purified and analyzed by mass spectometry, SDS PAGE, N-terminal sequencing, and C-terminal digestion. **A5099:13-19; A5066:16-20; TE-206; A5099:17-19; A5069:9-14; A5098:16-21.** Dr. Jorgensen found that the alpha-amylase in G997 was glycated, meaning that it had some sugar molecules attached to it. **A5081:15-21.** This does not affect the amino acid sequence of the protein. **A5085:10-13; A5081:15-21; A5089:10-5090:10; A5254:18-5255:10.**

134. The amino acid sequence of G997 determined by Dr. Jorgensen is shown in **TE-199, A8529; A5071:18-21.** That sequence has 486 amino acids and is reproduced below:

Sequence of G997

AAPFNGTMMQ YFEWYLPDDG TLWTKVANEA NNLSSLGITA LWLPPAYKGT SRSDVGYGVY
DLYDLGEFNQ KGTVRTKYGT KAQYLQAIQA AHAAGMQVYA DVVFDHKGGA DGTEWVDAVE
VNPSDRNQEI SGTYQIQAWT KFDFPGRGNT YSSFKWRWYH FDGVDWDESR KLSRIYKFRG
IGKAWDWEVD TENGNYDYLM YADLDMDHPE VVTELKNWGK WYVNTTNIDG FRLDAVKHIK
FSFFPDWLSY VRSQTGKPLF TVGEYWSYDI NKLHNYITKT NGTMSLFDAP LHNKFYTASK
SGGAFDMRTL MTNTLMKDQP TLAVTFVDNH DTEPGQALQS WVDPWFKPLA YAFILTRQEG
YPCVFYGDYY GIPQYNIPSL KSKIDPLLIA RRDYAYGTQH DYLDHSDIIG WTREGVTEKP
GSGLAALITD GPGGSKWMYV GKQHAGKVFY DLTGNRSDTV TINSDGWGEF KVNGGSVSVW
VPRKTT

135. Dr. Arnold reviewed Dr. Jorgensen's work as an expert for Novozymes. She is a Professor at California Institute of Technology with extensive experience in protein engineering. **A5132:21-5134:23.** Dr. Arnold found that, in her opinion, Dr. Jorgensen had analyzed an actual sample of G997 and had correctly determined its amino acid sequence. **A5166:25-5169:8.** She based her conclusion on the unrefuted facts that all of the amino acids from positions 34 through 486 were identical between Dr. Jorgensen's sequence in TE-199 and the G997 amino acid sequence from Genencor's interrogatory response; Dr. Jorgensen's G997 amino acid sequence and the stipulated Spezyme Ethyl amino acid sequence stop and start at the same places, and the

probability of a random protein sequence and Dr. Jorgensen's G997 amino acid sequence being exactly the same unless they were actually the same protein would be "very, very small." **A5168:15-24;** *see also* **A5166:25-5167:7.**

136. Dr. Arnold compared the admitted 484 amino acid sequence of Spezyme Ethyl (**TE-125, A8343**) to the 486 amino acid sequence of G997 determined by Dr. Jorgensen. (**TE-199, A8529**). She found that these two sequences are identical, except that the two amino acids at positions 179 and 180 of G997 are deleted in Spezyme Ethyl. **A5167:10-21.** She also concluded that "G997 is the parent from which the Spezyme Ethyl variant was derived." A5167:20-21.

137. At trial, Genencor agreed to provide Novozymes with an additional sample of G997 for analysis. **A5157:2-15.** Dr. Jorgenson received that sample from Genencor and conducted the same analysis as before to determine the amino acid sequence of its alpha-amylase. The amino acid sequence he determined is shown in **TE-226.** It is identical to the amino acid sequence shown in **TE-199**, which Dr. Jorgenson determined from the previous G997 sample. ***Compare* TE-226 and TE-199.** Genencor has stipulated, *inter alia*, that the sequence in TE-226 "is the sole amino acid sequence of the only alpha-amylase determined by Novozyme from its analysis to be present in the sample of EZYME G997 provided by Genencor." **A1721.**

138. The amino acid sequence encoded by the G997 gene is given at **TE-194, A8525.** This is not the actual sequence of the protein as obtained from cells which are used to produce it; nor is it a sequence determined by analysis of actual G997 samples. Instead, it is "the sequence of the [G997] protein encoded by the G997 alpha-amylase gene" **TE-194, A8525.** This is a predicted amino acid sequence, based on conceptually *ex vivo* reading of the known nucleotide (DNA) sequence of G997 and translating it into corresponding amino acids, using the genetic code as a guide (each nucleotide triplet of the coding DNA represents an amino acid). **A5164:5-17.** It is "not necessarily the protein sequence that you would actually isolate because the cells read the DNA in a particular way, and produce a protein according to how they read the DNA." ***Id.***

139. Genencor's proposed amino acid sequence for protein G997 has 514 or 515 amino acids, not 486 amino acids as determined by Dr. Jorgensen from actual samples of the enzyme. **A5209:7-17.**

140. The difference between a predicted amino acid sequence and the true sequence produced by a cell can be explained by a process called "post translational modification." **A5205:4-12.** When cells translate DNA to make preproteins, they also typically process the preprotein further to make a mature protein which is fully functional and ready for use. **A5204:18-5205:12.** *See e.g.* **A5265:19-25.**

141. In 2004, Genencor's Judy Chang did some work to compare and determine the amino acid sequences of EBS2 (Spezyme Ethyl) and G997. **TE-161, A8365-74.** She noted that G997 is a wild-type *B. stearothermophilus* alpha-amylase, produced by a wild-type gene. **A8365.** Further, EBS2 (Spezyme Ethyl) is genetically engineered from this wild-type. ***Id.*** It is a variant that differs from the wild-type by two amino acids at positions 179 and 180 (using *B. stearothermophilus* alpha-amylase (SEQ. ID NO. 3) for numbering). ***Id.***

142. The predicted amino acid sequence of G997 is given in Fig. 1 of the Chang report, which also indicates the two amino acids that are deleted to make Spezyme Ethyl. **TE-161, A8367.** This sequence shows 515 amino acids for G997 and 513 amino acids for Spezyme Ethyl, with predicted molecular weights of 58.7 kDa and 58.5 kDa, respectively, to account for the 179,180 deletion. **A8368; A5520:4-5522:11** (Dr. Alber). Chang noted that "However, the measured molecular weights … were ~3 kDa less than the theoretical molecular weight -- even for the wildtype G997 produced in *stearothermophilus*, indicating that the mature secreted protein is truncated." **A8368** (emphasis added).[3]

[3] A comparison was also made to Novozyme's Termamyl SC product (**A8368**), which is not relevant to this lawsuit.

143. Further sequencing work was done, using digest mapping, for "interrogation of the actual sequence for each protein." **A8368** (emphasis added). Peptide fragments resulting from the digest mapping did not include fragments corresponding to the 27-29 amino acid residues at the C-terminal ends of the predicted sequences. **A8368-9**. The Chang report concluded that, "a truncation of 27-29 amino acids [from the C-terminus] would be consistent with the molecular weights measured for the intact proteins." **A8369**.

144. Fig. 3 of the report shows a "suggested" truncation of 27 amino acids. ***Id.***

145. The Chang report indicates that the same truncation occurs in both the parent G997 and its variant Spezyme Ethyl. The parent and the variant are reported to differ from one another by the deletion at 179 and 180, not by how their C-termini are truncated. **A8368-9**.

146. Fig. 3 in the Chang report **(TE-161, A8369)** can be compared with the G997 sequence obtained by Dr. Jorgensen **(TE-199, A8529)**. Dr. Jorgensen's sequence is identical to the 29 amino acid C-terminus truncated amino acid sequence proposed by Chang. A8369. It is one of those suggested in the Chang report and is only two residues shorter than the suggested sequence in Chang's Fig. 3. (Jorgensen's sequence ends with "...VPRKTT," **(A8529)**, while Chang's Fig. 3 has two more amino acids, "...VPRKTTVS" **(A8369)**.

147. Dr. Jorgensen used precise methods for determining the amino acid sequence of G997. **A5066:16-20; TE-206; A5099:17-19; A5069:9-14; A5098:16-21**. He obtained the same results on different G997 samples tested at different times, confirmed the 29 amino acid truncation of Chang (Fact 149, *supra*), and found no evidence that other truncations occur or were present. **A5083:2-4; A5091:5-12; A5093:1-24.** His results are consistent with the Chang report, which proposed "a truncation of 27-29 amino acids." **A8369**.

148. Genencor's Dr. Alber agrees with the sequence determined by Dr. Jorgensen. "Except for the heterogeneity that Ms. Chang reported. We actually have no disagreement about the sequence of G997." **A5515:19-20**. Thus, Dr. Alber agrees that the sequence determined by Dr. Jorgensen is still present in the G997 samples (the 29 residue truncation), whether or not the

two other possibilities suggested by Ms. Chang (the 27 and 28 residue truncations) are also present. **A5514:16-5515:22.**

149. Genencor proffered no other experimental or sequencing work of its own or by anyone else. That Chang's work could not distinguish among three truncation possibilities (**A8369**) does not mean that all of them occur. *See,* Dr. Alber at **A5510:13-:22** (inconclusive); **A5511:9-19** (possible mixture). Jorgensen's work clarified the nature of the truncation and is not actually rebutted. There is no credible evidence to show that the amino acid sequence of G997 is anything other than the sequence determined and confirmed by Dr. Jorgensen.

150. Spezyme Ethyl is derived from, and is a variant of, G997, a wild-type *B. stearothermophilus* alpha-amylase. Conversely, G997 is the parent of Spezyme Ethyl.

151. Spezyme Ethyl has alpha-amylase activity. **TE-134, A8355; A5159:17-23.**

152. The two amino acids at positions 179 and 180 of its G997, "using SEQ. ID NO. 3 [of the patent] for numbering," are deleted in Spezyme Ethyl.

153. The amino acid sequence of Spezyme Ethyl (**TE-194, A8521-22; TE-125**) can be directly compared to the amino acid sequence of G997 determined by Dr. Jorgenson (**TE-199, A8529; TE-124; TE-226**). The evidence shows that when Spezyme Ethyl and G997 are aligned and percent homology is calculated according to the '031 specification and claims, Spezyme ethyl is at least 95% homologous to G997. **TE-126; A8347-48; A5113:22-5114:20** (Dr. Devereux); **A5158:16-5159:1** (Dr. Arnold).

154. Spezyme Ethyl has 484 amino acids (**A5167:8-17; TE-184; A8345 esp. 16-17**), and is the same as the amino acid sequence of G997 (**TE-199; A8529**), except for the two amino acids at positions 179 and 180 that are present in G997 and deleted in Spezyme Ethyl.

155. Dr. Devereaux, an expert for Novozymes, performed a sequence alignment between the Spezyme Ethyl sequence of **TE-125** and the G997 sequence of **TE-199**. He used version 10 of the GAP GCG program, with the default parameters from version 7 of the program as described in the patent. **TE-100, A7009 at 4:36-45**. The result, using the GAP formula for

percent identity, would be the same with GAP CGC version 7.3 or even with a pencil and paper calculation. **A5116:18-5117:1.**

156. An excerpt from this alignment report is reproduced below (**TE-126, A8347-8**).

```
Percent Similarity: 100.000   Percent Identity: 100.000

      Match display thresholds for the alignment(s):
                | = IDENTITY
                : =   2
                . =   1

G-997-2.pep x SPEZE.pep   November 17, 2005 15:30  ..

          .         .         .         .         .
    1 AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITALWLPPAYKGT 50
      ||||||||||||||||||||||||||||||||||||||||||||||||||
    1 AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITALWLPPAYKGT 50
          .         .         .         .         .
   51 SRSDVGYGVYDLYDLGEFNQKGTVRTKYGTKAQYLQAIQAAHAAGMQVYA 100
      ||||||||||||||||||||||||||||||||||||||||||||||||||
   51 SRSDVGYGVYDLYDLGEFNQKGTVRTKYGTKAQYLQAIQAAHAAGMQVYA 100
          .         .         .         .         .
  101 DVVFDHKGGADGTEWVDAVEVNPSDRNQEISGTYQIQAWTKFDFPGRGNT 150
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  101 DVVFDHKGGADGTEWVDAVEVNPSDRNQEISGTYQIQAWTKFDFPGRGNT 150
          .         .         .         .         .
  151 YSSFKWRWYHFDGVDWDESRKLSRIYKFRGIGKAWDWEVDTENGNYDYLM 200
      |||||||||||||||||||||||||||||  ||||||||||||||||||
  151 YSSFKWRWYHFDGVDWDESRKLSRIYKF..IGKAWDWEVDTENGNYDYLM 198
          .         .         .         .         .
  201 YADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDAVKHIKFSFFPDWLSY 250
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  199 YADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDAVKHIKFSFFPDWLSY 248
              .         .         .         .         .
  251 VRSQTGKPLFTVGEYWSYDINKLHNYITKTNGTMSLFDAPLHNKFYTASK 300
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  249 VRSQTGKPLFTVGEYWSYDINKLHNYITKTNGTMSLFDAPLHNKFYTASK 298
          .         .         .         .         .
  301 SGGAFDMRTLMTNTLMKDQPTLAVTFVDNHDTEPGQALQSWVDPWFKPLA 350
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  299 SGGAFDMRTLMTNTLMKDQPTLAVTFVDNHDTEPGQALQSWVDPWFKPLA 348
          .         .         .         .         .
  351 YAFILTRQEGYPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH 400
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  349 YAFILTRQEGYPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH 398
          .         .         .         .         .
  401 DYLDHSDIIGWTREGVTEKPGSGLAALITDGPGGSKWMYVGKQHAGKVFY 450
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  399 DYLDHSDIIGWTREGVTEKPGSGLAALITDGPGGSKWMYVGKQHAGKVFY 448
              .         .         .
  451 DLTGNRSDTVTINSDGWGEFKVNGGSVSVWVPRKTT 486
      |||||||||||||||||||||||||||||||||||||
  449 DLTGNRSDTVTINSDGWGEFKVNGGSVSVWVPRKTT 484
```

157. This alignment confirms that the two sequences are identical, except for the amino acids RG present at positions 179 and 180 of G997 (the top sequence), but deleted in Spezyme Ethyl (the bottom sequence). **A5167:8-17.**

158. The GAP GCG program calculated the percent identity between the two sequences as 100%. **TE-126, A8347**; **A5113:22-5115:20** (Dr. Devereaux); **A5158:18-5159:1** (Dr. Arnold). All 484 amino acids of Spezyme Ethyl are present in the 486 amino acid sequence of G997. **TE-126, A8347-48.** Thus, all (100%) of the Spezyme Ethyl amino acid sequence is present in the G997 amino acid sequence.

159. All 486 amino acids of G997 can not be present in the shorter 484 amino acid sequence of Spezyme Ethyl. Nevertheless, if a hypothetical percent comparison is made in this way, and if the two amino acid gap in Spezyme Ethyl is counted, the result is 484/486 x 100% = 99.59%. **A5256:13-22.** Spezyme Ethyl would still be "at least 95% homologous" to G997.

160. Assuming hypothetically that G997 has the 488 amino acid sequence suggested in Fig. 3 of the Chang report (**TE-161, A8369**), and again assuming that gaps should be counted for percent calculations, and although this is different from the standard calculation performed by the GAP GCG program, the result would be 484/488 x 100% = 99.2%.

161. The presence of additional unmatched amino acids at the C-terminus of G997 would not change the percent identity calculated by GAP CGC. **A5191:6-15** (Dr. Arnold). All of the Spezyme Ethyl sequence would still be present in the G997 sequence. ***Id.***

162. Dr. Devereaux also compared the Spezyme Ethyl sequence with SEQ. ID NO. 3 of the '031 patent, using the GAP GCG program, with default parameters from version 7.3 according to the patent. **TE-127, A8349-50; TE-100, A7009 at 4:36-45.** *See also,* **A5116:18-5117:1.**

163. A percent identity of 98.967% was calculated for the alignment between Spezyme Ethyl and SEQ. ID NO. 3. **TE-127, A8349.** *See also,* **A5118:22-5119:16** (Dr. Devereaux); **A5160:7-15; A5161:7-12** (Dr. Arnold).

164. An excerpt from this alignment report is reproduced below (**TE-127, A8349-50**).

```
Percent Similarity: 99.380   Percent Identity: 98.967

      Match display thresholds for the alignment(s):
                  | = IDENTITY
                  : =   4
                  . =   1

NewB.pep x SPEZE.pep      June 3, 2005 11:08  ..

      1 AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITALWLPPAYKGT 50
        |||||||||||||||||||||||||||||||||||||||||||||||||||
      1 AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITALWLPPAYKGT 50

     51 SRSDVGYGVYDLYDLGEFNQKGAVRTKYGTKAQYLQAIQAAHAAGMQVYA 100
        |||||||||||||||||||||||:|||||||||||||||||||||||||||
     51 SRSDVGYGVYDLYDLGEFNQKGTVRTKYGTKAQYLQAIQAAHAAGMQVYA 100

    101 DVVFDHKGGADGTEWVDAVEVNPSDRNQEISGTYQIQAWTKFDFPGRGNT 150
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    101 DVVFDHKGGADGTEWVDAVEVNPSDRNQEISGTYQIQAWTKFDFPGRGNT 150

    151 YSSFKWRWYHFDGVDWDESRKLSRIYKFRGIGKAWDWEVDTENGNYDYLM 200
        |||||||||||||||||||||||||||||  ||||||||||||||||||||
    151 YSSFKWRWYHFDGVDWDESRKLSRIYKF..IGKAWDWEVDTENGNYDYLM 198

    201 YADLDMDHPEVVTELKSWGKWYVNTTNIDGFRLDAVKHIKFSFFPDWLSD 250
        |||||||||||||||.|||||||||||||||||||||||||||||||||||
    199 YADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDAVKHIKFSFFPDWLSY 248

    251 VRSQTGKPLFTVGEYWSYDINKLHNYIMKTNGTMSLFDAPLHNKFYTASK 300
        |||||||||||||||||||||||||||| ||||||||||||||||||||||
    249 VRSQTGKPLFTVGEYWSYDINKLHNYITKTNGTMSLFDAPLHNKFYTASK 298

    301 SGGTFDMRTLMTNTLMKDQPTLAVTFVDNHDTEPGQALQSWVDPWFKPLA 350
        |||:||||||||||||||||||||||||||||||||||||||||||||||||
    299 SGGAFDMRTLMTNTLMKDQPTLAVTFVDNHDTEPGQALQSWVDPWFKPLA 348

    351 YAFILTRQEGYPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH 400
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
    349 YAFILTRQEGYPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH 398

    401 DYLDHSDIIGWTREGVTEKPGSGLAALITDGPGGSKWMYVGKQHAGKVFY 450
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
    399 DYLDHSDIIGWTREGVTEKPGSGLAALITDGPGGSKWMYVGKQHAGKVFY 448

    451 DLTGNRSDTVTINSDGWGEFKVNGGSVSVWVPRKTTVSTIAWSITTRPWT 500
        ||||||||||||||||||||||||||||||||||||
    449 DLTGNRSDTVTINSDGWGEFKVNGGSVSVWVPRKTT.............. 484
```

165. This alignment confirms that that Spezyme Ethyl (bottom row) does not include the two amino acids, R and G, at positions 179 and 180 of SEQ. ID NO. 3 (top row). Thus, when SEQ. ID NO. 3 is used as a reference for numbering purposes, the amino acids at positions 179 and 180 are deleted. A comparison of **TE-126** and **TE-127** shows that G997 and SEQ. ID NO. 3

have the same numbering. The same two amino acids are at positions 179 and 180 of G997, when SEQ. ID NO. 3 is used for numbering. **A5119:22-5120:7** (Dr. Deveraux); **A5159:2-14** (Dr. Arnold); **A5162:9-2**

D. Validity

1. The Prior Art/Obviousness

166. The relevant field for the invention claimed in the '031 patent is protein engineering. A person of ordinary skill in this art has a Ph.D. in molecular biology or a related field, has done protein engineering in the lab, and has written and read papers in the field. **A5202:3-10** (Dr. Alber).

167. Genencor relies, *inter alia*, on two publications cited by the PTO to reject claims during prosecution of the '031 patent: (i) an article published in the November 15, 1989 issue of the Journal of Biological Chemistry by Yutaka Suzuki *et al.*, titled "Amino Acid Residues Stabilizing a Bacillus α [Alpha]-Amylase against Irreversible Thermoinactivation," ("Suzuki") (**TE-115**); and (ii) International Patent Publication No. WO 95/10603 by Bisgård-Franzen *et al.*, titled "Amylase Variants" and dated April 20, 1995 ("Bisgard-Frantzen") (**TE-177**). **A1007, ¶AA.**

(a) Suzuki (TE-115)

168. Suzuki describes mutants or variants of the alpha-amylase enzyme of *Bacillus amyloliquefaciens* ("BAN"). It says that some of these mutants have improved thermostability compared to the wild-type *B. amyloliquefaciens* alpha-amylase from which they were derived. **A1007, ¶BB.**

169. Suzuki discloses that: "[t]he [alpha]-amylase of *Bacillus licheniformis* (BLA) is stable and active at high temperature. More than 80% of its activity is retained after heat treatment at 90°C for 30 min, and the optimum temperature for its activity is 80-85°C. In contrast, the [alpha]-amylase of *Bacillus amyloliquefaciens* (BAA), [a.k.a. BAN], the amino acid sequence of which is 80% homologous with that of BLA, is rapidly inactivated at 90°C." **TE-115, A8233**

(Abstract). Based on experiments with BAA mutants, "two regions in the amino acid sequence of BLA comprising Gln^{178} (region 1) and the 255^{th}-270^{th} residues (region II), respectively, were shown to determine the thermostability of BLA. Region I plays a major role in determining the thermostability." ***Id.***

170. More specifically: "deletion of Arg^{176} and Gly^{177} in region I and substitutions of alanine for Lys^{269} and aspartic acid for Asn^{266} in region II were shown to be responsible for the enhancement of thermostability." ***Id.*** In Suzuki's experiments, "[m]utant BAAs containing the above deletions and substitutions showed almost the same thermostability as BLA as to irreversible thermoinactivation. Nevertheless, the mutant BAAs showed a temperature optimum as low as that of BAA (65°C), indicating they are still susceptible to reversible inactivation at temperatures higher than 65°C." **TE-115, A8233**.

171. Region I of Suzuki was further identified as follows: "the amino acid sequence of BLA from the 177^{th} to the 186^{th} positions should play a major role in the enhancement of the thermostability of the enzymes. This region was designated as region I." **TE-115, A8236-8237**. When BAA is modified to match an optimal alignment with BLA, so that region I Arg^{176} and Gly^{177} of BAA are deleted and Glu^{178} is changed to Gln^{178} (the "BAA176-178**Q mutant") and the region II Lys^{269} of BAA is also changed to the Ala^{269} found in BLA, the modified BAA enzyme is "almost as thermostable as BLA." ***Id.*** **at A8237.** Suzuki suggests that "the deletion of arginine and glycine in this region is important for enhancement of the thermostability of the [BAA] enzyme." ***Id.*** Also, "In region I, consecutive deletion of Arg^{176} and Gly^{177} seemed to be important for enhancement of the thermostability [of BAA]." ***Id.*** **at A8238.** The residues Arg and Gly can also be represented by single-letter abbreviations R and G, respectively.

172. Suzuki discloses further that the nucleotide sequences for alpha-amylase genes from BAA, BLA, and BSG had been determined. Based on this work, the amino acid sequence of BLA was described as 80% homologous to BAA and 65% homologous to BSG. **TE-115, A8233.** Despite this resemblance in primary structure, Suzuki states that "the three enzymes show

diversity in heat and pH stability; the thermal stability dramatically increases in the order of *B. amyloliquefaciens*, *B. stearothermophilus*, and *B. licheniformis* alpha-amylases." ***Id.***

173. Suzuki suggests two ideas to explain how the amino acid alterations he studied might work. "These alterations might enhance the thermostability by influencing the electrostatic interaction within the enzyme molecule. Alternatively, they may enhance the thermostability by improving the internal packing arrangements of enzymes since these alterations also lead to an increased hydrophobicity." **TE-115, A8238.**

174. Suzuki tested BLA and BAA under heat inactivation conditions that included a buffer containing "10 mM calcium ($CaCl_2$) prewarmed at 90°C." **TE-115, A8234.**

175. Suzuki identifies amino acid deletions that improve the thermostability of BAA relative to BLA. Suzuki does not disclose any mutants or variants of a *B. stearothermophilus* alpha-amylase. Suzuki does not make any experimental comparisons of a wild-type *B. stearothermophilus* alpha-amylase to any altered or modified *B. stearothermophilus* alpha-amylases, nor to any BAA or BLA wild-type or mutant alpha-amylases. Suzuki does not make any predictions concerning the thermostability of *B. stearothermophilus* alpha-amylase or mutants. **A5721:21-5722:5; A6527:19-6530:2.**

(b) Bisgard-Frantzen (TE-177)

176. Bisgard-Frantzen described alpha-amylase enzymes from three *Bacillus* species: *B. amyloliquefaciens* ("BAN"), *B. stearothermophilus* ("BSG"), and *B. licheniformis* ("BLA") and taught that these alpha-amylases have highly homologous amino acid sequences. **A1007, ¶CC.**

177. Bisgard-Frantzen acknowledges Suzuki (**TE-177, A8406**) and discloses different alpha-amylase variants having improved thermostability, which are not at issue in this litigation.

178. Bisgard-Frantzen provides an alignment of the amino acid sequences of BLA (SEQ. ID NO. 2); BAN (SEQ. ID NO. 4), and BSG (SEQ. ID NO. 6). **TE-177, A8413, A8415-6.** In this alignment, the residues R and G at positions 176 and 177 of BAN in SEQ. ID NO. 4 of the

reference correspond to the residues R and G at positions 179 and 180 of BSG in SEQ. ID NO. 6. **A8415.**

(c) The PTO Rejection

179. During the prosecution of the '031 patent, the PTO examiner issued an obviousness rejection under 35 U.S.C. § 103(a) based on a combination of Suzuki and the Bisgard-Frantzen PCT. **A1007.** The PTO Examiner stated that "it would have been obvious to one of ordinary skill in the art to introduce the mutations disclosed by Suzuki *et al.* into the corresponding positions of *Bacillus stearothermophilus* [alpha]-amylase in order to produce a homologous [alpha]-amylase which would have been reasonably expected to have similar improved properties in view of the known homology between these [alpha]-amylases." **A1008, ¶EE.**

180. Novozymes overcame the obviousness rejection by submitting a Declaration under 37 C.F.R. § 1.132 from Dr. Borchert (the "Borchert § 132 Declaration"). **A1008**; ¶FF *see also* **TE-508., A8857.**

(d) The Borchert Declaration

181. The Borchert Declaration **(TE-508, A8857-8874)** was submitted to show that deleting the amino acids at positions 179 and 180 of BSG led to unexpected results, relative to the deletion disclosed by Suzuki for BAN. **A5640:19-5641:6.** Thus, "an experiment was carried out comparing the deletion of R179-G180 in *B. stearothermophilus* alpha-amylase (BSG) to the deletion of R176-R177 in *B. amyloliquefaciens* alpha-amylase (BAN)." **TE-508, A8857 at ¶3.** "[T]he variants were otherwise identical to the wild-type alpha-amylases." **TE-508, A8858 at ¶4.**

182. Cells producing the wild-type and mutant alpha-amylases were grown under identical conditions, and the respective alpha-amylases were recovered in order to test for residual activity by thermal inactivation "carried out at 80 degrees Celsius." **TE-508, A8858 at ¶5.** "The temperature of 80 degrees Celsius was chosen as the highest temperature where both BAN and BSG wild-type and derived variants could be reliably compared." ***Id.*** The samples were heat-

treated in a PCR machine (***Id.***), and the test conditions included "0.1 mM calcium". **A8859, ¶5.** *See also*, **A6093:15-18.**

183. Measurements of the residual activity of each alpha-amylase sample at various times are reported in the Declaration. **TE-508, A8859 at ¶6.** "For BSG wild-type and BSG variant two series of experiments were carried out due to the necessary, long incubation times" (***Id.***). Resulting data is provided in a Table. **TE-508, A8859-60.** The half-life of thermal inactivation for each enzyme was calculated from the data using regression analysis. ***Id.*, A8860, ¶7.** The results are shown graphically in a semi-logarithmic plot of residual activity versus time. ***Id.*, A8861, ¶8.**

184. From these experiments, Dr. Borchert concluded (**TE-508, A8861-8862, ¶9**):

> The deletion of R179-G180 in BSG has a pronounced and very surprising effect on the thermal stability in BSG as compared to the deletion of R176-G177 in BAN. The deletion of R179-G180 in BSG causes a 63 fold increase in half-life at 80 degrees Celsius, whereas the deletion of R176-G177 in BAN causes only an 11 fold increase in half life at the same conditions. The deletion of R179-G180 in BSG gives a relative improvement of thermal stability which is 5 to 6 times higher than what is seen in BAN having the deletion of R176-G177. These results are statistically significant and very surprising as the effect of the double deletion in BSG is significantly greater than what would have been expected based on the combined teachings of Suzuki … in view of Bisgard-Frantzen. … The statistical analysis is attached as Appendix 1.

185. Genencor has not submitted any experiments or data comparing the thermostability or thermal inactivation properties of any alpha-amylase enzymes or variants and, in particular, comparing the thermostability of BSG and its 179,180 deletion variant to the thermostability of BAN and its 176,177 deletion variant. **A6550:10-11.**

186. When Dr. Borchert designed the experiments reported in his declaration, he planned to evaluate what effect the deletion of two amino acids have in BAN and BSG. **A5585:10-11.** He set out to do a fair and reliable comparison of the effect this deletion has on thermostability. **A5640:19-5641:1; A6092:11-14.** He made this comparison in view of the comments made by the PTO examiner and because the BAN deletion of Suzuki was believed to be the closest prior art to the claimed BSG variants of the '031 patent. **A5641:2-9.** Dr. Borchert

believed that BSG would be stabilized by these deletions, but he did not have any idea of how much it would be stabilized. **A5461:10-19**.

187. The alpha-amylase variants claimed in the '031 patent are intended for industrial use, and industrial enzymes should be tested under applicable industrial conditions. **A5052:9-13**. The applicable industrial conditions for these enzymes include elevated temperatures, *e.g.*, 80-90 degrees Celsius, and low calcium concentration; *e.g.*, 0.1 mM. **A5025:10-15; A5026:25-5027:10; A6092:15-25; A6534:7-14; A6536:2-11.**

188. A particularly important property for the industrial application of these enzymes is thermostability. **A6534:7-14; A5025:10-15.** One test for thermostability is called the half life of thermal deactivation. This is a measure of the amount of time needed for an enzyme to lose half of its activity under given temperature conditions. **A5788:1-4** (Dr. Klibanov). Dr. Borchert compared wild-type BAN with a variant having Suzuki's deletion of amino acids 176 and 177 ("BAN delete"). He did the same for BSG wild-type and a BSG variant having the deletion of amino acids 179 and 180 ("BSG delete"). **A5793:12-5794:8**. He measured the thermal inactivation of these four enzymes; *i.e.*, how much of their activity is left after heating for various periods of time. **A5787:11-13.**

189. Dr. Borchert employed a common method for testing the thermostability of an enzyme, which is to place the enzyme in a buffer solution in a predetermined temperature bath, to take samples at different times, and to measure residual activity. **A5052:23-5053:12; A5618:8-5619:22; A5786:14-5787:13**.

190. Dr. Borchert's lab technician, Ms. Holbo, carried out these experiments taking two separate measurements at each time point. **A5808:14-16** (Dr. Klibanov); **A6076:6-8** (Holbo). Ms. Holbo has worked for Dr. Borchert for 11 years and has been a technician at Novozymes for over 30 years. **A5641:24-25; 5642:1-3**. She then gave her data to a chemist experienced in statistics, Dr. Thomas Paulsen, for statistical analysis. **A6076:9-20**. Dr. Paulsen conducted a statistical analysis and provided results to Dr. Borchert in the form of graphs. Those graphs were

submitted to the PTO as an attachment to the Borchert Declaration. **A5598:4-13; A5616:12-16, 22-25; A5642:13-5643:1.**

191. As reported in the Declaration, Dr. Borchert found a 63-fold increase in thermostability for the '031 patent's BSG delete variant over the parent BSG. He found only an 11-fold improvement in thermostability for the Suzuki BAN delete variant over the parent BAN. **TE-508, A8861-8862, ¶9; A6532:11-6533:14** (Dr. Arnold). A 63-fold increase is typically very significant. **A6009:2-16** (Dr. Klibanov). It is also appropriate to compare the improvements in BSG and BAN as Dr. Borchert did, by taking "the ratio of those two 63 over 11, and I believe that comes out to 5.7." **A6533:16-6534:1** (Dr. Arnold). *See also,* **A5819:16-21**) (Dr. Klibanov). This means that the stabilizing effect of the 179,180 deletion in BSG is about 5.7 times greater than that for the same deletion in BAN. **A6533:16-6534:1.**

192. These results show that, in a practical industrial sense, BSG delete is still active after 2840 minutes (over 2 days) and still retains 50% of its activity after 3 days at 80° C. **A6534:21-6535:4.** On the other hand, BSG wild-type loses most of its activity after only 2 hours. **A6535:9-16.** Dr. Arnold summarized this simply as "So we're talking hours vs. days." **A6535:16.** This has an important effect in a commercial process such as the fuel ethanol process. **A6535:17-19.** This is in further contrast to the half life of the Suzuki wild-type and variant BAN enzymes, which is less than 10 minutes for both of them. **TE-508; A8860 at ¶7.**

193. Dr. Borchert compared the half-life of thermal inactivation of these BAN and BSG enzymes and variants at 80°C. This is an appropriate elevated temperature for such a comparison, particularly in the context of the industrial conditions used in fuel ethanol production. **A6536:12-6537:25; A5025:3-7, 8-15; A6534:7-14.** It "was a fair temperature to choose." **A6536:24-25.**

194. Additionally, calibration studies showed that this was a temperature at which all four enzymes could be compared. **A6076:21-6077:4.** A higher temperature inactivated BAN too quickly for a meaningful comparison to be made with BSG. **A6095:23-6096:6.** At a temperature

lower than 80° C, it would have taken weeks to see any kind of decay in BSG delete, the most thermally stable of the four. **A6096:7-12.** The calibration experiments showed that 80° C was the temperature at which all four enzymes could reasonably be compared, from the least thermostable (BAN wild type) to the most thermostable (BSG delete). **A6094:8-14; A6095:12-21.**

195. One temperature was used because all four enzymes needed to be tested under identical conditions, in order to make a fair and proper comparison. **A6094:15-17; A6096:13-17; A6536:12-19.**

196. Dr. Borchert compared the half-life of thermal inactivation of these BAN and BSG parents and variants using a calcium concentration of 0.1 mM. This is an appropriate calcium concentration for such a comparison, particularly in the context of enzymes to be used in fuel ethanol production. **A5026:1-5027:14; A5029:12-20; A6092:15-25.** Additionally, it was a specifically stated goal of the invention, set forth in the patent, to provide variants having improved (*i.e.*, decreased) calcium dependency. **TE-100**; **A7008 at 2:61-3:1, esp. 2:66.** *See also, Id.;* **A7012 at 10:40-49**. The patent specifically recites 0.1 mM calcium as a targeted calcium level. **A6092:23-25; A7022 at 30:62-67; A6536:2-7.**

197. Dr. Borchert did not use the same 10 mM calcium concentration as Suzuki (**A5627:18-21**) because Suzuki's calcium concentration is extremely high and was not commercially relevant. **A6114:18-20.**

198. Genencor's Dr. Zeikus testified that he would not use the much higher Suzuki calcium concentration if he were running such comparison experiments. **A6114:18-24.** He also testified that Suzuki's calcium level alone would increase the thermostability of BAN wild-type vs. BAN delete by 12 or 13 fold, as opposed to the 11 fold increase reported by Dr. Borchert. **A6111:19-22.** Such a high calcium concentration may also have increased the 63 fold improvement reported by Dr. Borchert for the BSG delete variant. **A6114:1-18.**

199. Like Dr. Borchert, Genencor's Dr. Machius used 0.1 mM calcium in his papers and in other thermal stability studies. **A5732:8-20.**

200. Dr. Borchert did not use a pre-heated buffer solution in his experiments. **A5628:4-7.** When used, pre-heating is intended to address the possibility of a ramp-up time; *i.e.* the time it takes a sample to reach the targeted experimental temperature, here 80° C. **A5796:12-14** (Dr. Klibanov). If the ramp-up time is too long, the first measurements of residual activity for short-lived enzymes (*e.g.* BAN) may be less precise, because the sample will be at the targeted temperature for a period of time slightly shorter than is reported. **A5796:5-10** (Dr. Klibanov).

201. Dr. Borchert did not preheat, because he used a PCR machine preheated to 80° C to heat the samples. **A6078:12-15.** Very thin 150 microliter PCR tubes were used for this assay. **A6075:5-14; TE-215.** It is a standard protocol to proceed without preheating the sample when using a PCR machine or a thermocylcer. **A6538:17-21.** Pre-heating is not an issue at all with the longer half-life alpha-amylases (e.g. BSG). **A6012:13-17** (Dr. Klibanov).

202. The 150 microliter PCR tubes are capped with a snap on lid. **A6075:5-14; TE-215.** These tubes may have evaporation problems when they are used in a PCR machine for long incubation periods. **A6077:5-9.** This is why an additional set of samples was incubated in a heat oven, using different tubes with a tighter screw-cap. **A6077:13-6078:4; TE-214.**

203. Genencor's Dr. Klibanov speculated from hypothetical data that ramp-up time might have had an effect on Dr. Borchert's experiment. **A6014:17-23.** Although BAN and BSG are available today and Dr. Klibanov could have conducted experiments, he did not do any actual experiments, such as comparing protocols with and without a preheated buffer. **A6005:1-6006:6; A6006:16-6007:12; A6018:16-22.**

204. Dr. Arnold, on the other hand, had Dr. Tams at Novozymes run experiments to determine the effect, if any, of ramp-up time. **A6537:12-19.** Dr. Tams compared the thermal inactivation rate of the enzymes in preheated and non-preheated buffers. **TE-208R; A6543:14-6544:2.** The protocols and results of these experiments were entered into standard Novozymes lab notebooks. **A6045:3-25; A6046:17-6047:2; A6047:3-6050:11; TE-218.** These experiments were run to determine the rate of inactivation at 80° C of BAN wild-type using preheated and

non-preheated buffers in 250 microliter PCR or thermocycler tubes. **A6041:17-20; A6042:1-4; A6059:2-25; A6060:3-7; TE-223.**

205. Dr. Tams' results show that "you get essentially the same answer, whether it's preheated or not." **A6544:3-11.** Although a 15-20% difference can be observed, "[t]hat difference is not significant." **A6544:18-19.** "They're very close." **A6544:21.** This is not Dr. Klibanov's hypothetical three-fold effect. **A6544:12-14.** The actual ramp-up experiments run by Novozymes and reviewed by Dr. Arnold showed that "the ramp-up time had essentially no effect." **A6545:22-25.** The conclusions from Dr. Borchert's experiments do not change and do not depend on whether or not the buffer was pre-heated.

206. Genencor recently filed a patent application **(TE-202, A8532.1)** directed to *B. stearothermophilus* alpha-amylase enzymes. The alpha-amylases claimed in this application are the same '031 patent 179,180 deletion variants at issue in this case. Genencor's application includes experiments to measure the thermal inactivation rates of these enzymes. **A6538:24-6539:4; A6539:23-6540:3.** Genencor uses a hot water bath or a hot block for heating the enzyme. There is no instruction to preheat the buffer in the protocol used by Genencor. **A6540:20-25.** Rather, Genencor, just took "the little itty-bitty Eppenedorf tubs [*sic*:tubes] … and put them into the hot block that has been preheated. That is exactly what [Novozymes'] Ms. Holbo did." **A6540:25-6541:3** (Dr. Arnold). Both Genencor and Novozymes use protocols without preheating the buffer; this is an acceptable standard in the industry. **A6541:12-19.**

207. Six potential data points in the raw results obtained by Ms. Holbo were not reported in the Borchert Declaration and were not used in the calculations. **A5807:20-5808:1.** These were one of each of the two data points for BSG wild-type at 20 and 40 minutes **(A5810:15-812:6)**; two data points for BSG delete at 2881 minutes **(A5808:1-2)**; and both data points for BSG delete at 2940 minutes **(A5809:22-5810:3)**. These data points were properly excluded because they were unreliable. This is what any competent scientist would have reasonably done. **A5614:25-5615:16; A5615:22-5616:2.**

208. Ms. Holbo carried out the experiments in duplicate. **A5808:14-16** (Klibanov); *See also*, **TE-508, A8859 at ¶6** (double determination for each time point). The residual alpha-amylase activity of each sample of enzyme, incubated for a given period of time, was measured twice. **A6547:8-12** (Arnold). A reason to run duplicates is that if the sample is good, but one of the readings is unreliable, the data point for the other reading can be used. **A5768:11-15** (Klibanov).

209. Two of the samples, one at 20 and one at 40 minutes, were clouded by solid material and could not be reliably used. The color spectrometry assay used by Ms. Holbo can be adversely affected by loose (undissolved) enzyme substrate in the sample, which can be withdrawn with the liquid that is taken out of the test tube for the enzyme activity reading. If the liquid withdrawn from the sample for a reading contains solid material, the optical (colorimetric) measurement of enzyme activity can be inaccurate. **A6080:3-14.** When Ms. Holbo sees loose substrate, she puts brackets around the measurement indicating that the assay of this sample is suspect. ***Id.*** She observed this problem with one of the two readings for the sample assayed at 20 minutes and with one of the two readings for the sample assayed at 40 minutes. **A5811:15-5812:16** (Klibanov). Therefore, one data point at each of these times was properly omitted from the statistical analysis. The sample itself was not questioned; the other two data points, one at 20 minutes and one at 40 minutes, were not clouded by substrate, and these latter two points were properly used in the analysis. ***Id.*** (Klibanov).

210. The sample at 2881 minutes was not used because Ms. Holbo's visual inspection of this sample showed evaporation when she removed it from the PCR machine. An evaporated sample is defective. Although she dutifully made two readings of this sample, she properly told the statistician not to use this data because of the evaporation and because she believed that the readings were unreliable. **A6079:5-14**; **A5643:17-24**. Evaporation is a recognized risk in such experiments (**A6077:5-9**), and it is standard procedure in scientific laboratories to exclude unreliable data, such as data from a sample which inspection shows has lost its integrity, *e.g.*,

because of evaporation. Since the sample itself was defective, neither reading at 2881 minutes could be used. **A6549:7-20** (Dr. Arnold); **A6020:14-24** (Dr. Klibanov).

211. Dr. Borchert excluded both measurements for BSG at 2940 minutes. **A5643:25-5644:2**. One of the readings showed greater than 130% residual activity after 2940 minutes (more than 2 days of heat). **A5645:8-12** Earlier time-point readings showed a progressive loss of activity. The enzyme could not be gaining more activity than it started with, particularly after so much time at a high temperature. **A5809:22-5810:2**. Genencor's Dr. Klibanov agreed that such readings greater than 100% make no sense. ***Id.*** The reading of the other sample at 2940 minutes showed a residual activity as low as 56%. **A5645:12-13**. Dr. Borchert left out both points because there was a 120-130% difference between the two readings. Given this very wide discrepancy, the sample was suspect, and neither point could properly be relied on. **A5645:8-17; A6546:14-A6548:5**.

212. Dr. Arnold concluded that it was "a reasonable decision" not to include the 2940 minute data points because the higher data point "didn't make any biological sense," and the lower data point would result in improper distortion of the data **A6546:1-12, 24-25; A6547:20-6548:5**. Actually, if both points had been left in, the difference between BSG delete and BSG wild-type would increase from the reported 63 fold improvement to a 77 fold improvement. **A6548:8-16**. If Dr. Borchert had excluded the higher data point, but included the lower one, the improvement of BSG delete over BSG wild-type would still be 55 fold. **A6548:17-25.** Thus, "[t]he whole range of all the possibilities of those two data points takes the relative improvement from 55 to 77. **A6549:4-6**. "This 55 to 77 fold effect could not be predicted." **A6550:16-22**.

213. The judgment to exclude doubtful data is good scientific method. **A5644:3-5645:7**. The Borchert Declaration, including the reported data and considering the discounted readings, is "a fair representation of what is actually going on with these enzymes." **A6550:1-9**. Dr. Arnold added that she has not seen any counter data from Genencor, and "if Borchert's data

were far from the truth, I think that I would have seen those data from some other place." **A6550:13-15.**

214. When a thermal inactivation experiment produces readings that are before and after the half-life, the half-life itself is "interpolated." This is a routine determination, and is done because half life is not measured directly. The odds of sampling at precisely the time that turns out to be the half life (the 50% activity point) are very slim. **A5789:8-24.** When no sample is read after the half life, the half life can be "extrapolated" from earlier data points. This was the case for the BSG delete variant. The thermostability was so improved that, even after the last sample taken at 4200 minutes (almost three days), BSG delete still had a residual activity of 61% (more than 50%). **TE-508, A8858**; **A6535:3-8** (variant enzyme keeps going after days at 80 °C). Thus, Novozymes' Dr. Paulsen used extrapolation to determine the half life. This approach takes the line formed by the points before the half life and extends the line to reach the later half-life point. **A5803:10-22.**

215. Genencor's patent application, discussed above, included claims to the same variants that are at issue here. **A6542:25-6543:5** In that application, Genencor determined the half life of the enzymes that it studied in the same way that Novozymes did. "[T]he way they [Genencor] process the data in this protocol [in Genencor's patent application] is exactly the same way, exactly the same way that Dr. Paulson processed Ms. Holbo's data. They [Genencor] took the natural log or the log of the residual activity and drew a straight line through those data points." **A6542:10-14.**

(e) <u>The Machius Reference</u>

216. Genencor has relied on an additional reference: Machius et al., J. M. Biol. (1995) 246, 545-559 ("Machius '95"). **TE-173, A8375-8390.**

217. In Machius '95, Dr. Machius studied only *Bacillus licheniformis* (BLA) through X-ray crystallography. **A5635:21-24.**